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(57) Abstract An isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant.		

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Methods for Obtaining Plant Varieties

TECHNICAL FIELD

The present invention relates to nucleotide sequences which encode polypeptides involved in the DNA mismatch repair systems of plants, and to the polypeptides encoded
5 by those nucleotide sequences. The invention also relates to nucleotide sequences and polypeptide sequences for use in altering the DNA mismatch repair system in plants. The invention also relates to a process for altering the DNA mismatch repair system of a plant cell, to a process for increasing genetic variations in plants and to processes for obtaining plants having a desired characteristic.

BACKGROUND OF THE INVENTION

10 Plant breeding essentially relies on and makes use of genetic variation which occurs naturally within and between members of a family, a genus, a species or a subspecies. Another source of genetic variation is the introduction of genes from other organisms which may or may not be related to the host plant.

15 Allelic loci or non-allelic genes which constitute or contribute to desired quantitative (e.g. growth performance, yield, etc.) or qualitative (e.g. deposition, content and composition of seed storage products; pathogen resistance genes; etc.) traits that are absent, incomplete or inefficient in a species or subspecies of interest are typically introduced by the plant breeder from other species or subspecies, or *de novo*. This
20 introduction is often done by crossing, provided that the species to be crossed are sexually compatible. Other means of introducing genomes, individual chromosomes or genes into plant cells or plants are well known in the art. They include cell fusion, chemically aided transfection (Schocher et al., 1986, Biotechnology 4: 1093) and ballistic (McCabe et al., 1988, Biotechnology 6: 923), microinjection (Neuhaus et al., 1987, TAG 75: 30),
25 electroporation of protoplasts (Chupeau et al., 1989, Biotechnology 7: 53) or microbial transformation methods such as Agrobacterium mediated transformation (Horsch et al., 1985, Science 227: 1229; Hiei et al., 1996, Biotechnology 14: 745).

However, when a foreign genome, chromosome or gene is introduced into a plant, it will often segregate in subsequent generations from the genome of the recipient plant or
30 plant cell during mitotic and meiotic cell divisions and, in consequence, become lost from the host plant or plant cell into which it had been introduced. Occasionally, however, the introduced genome, chromosome or gene physically combines entirely or in part with the genome, chromosome or gene of the host plant or plant cell in a process which is called recombination.

35 Recombination involves the exchange of covalent linkages between DNA molecules in regions of identical or similar sequence. It is referred to here as homologous recombination if donor and recipient DNA are identical or nearly identical (at least 99%

base sequence identity), and as homeologous recombination if donor and recipient DNA are not identical but are similar (less than 99% base sequence identity).

The ability of two genomes, chromosomes or genes to recombine is known to depend largely on the evolutionary relation between them and thus on the degree of sequence similarity between the two DNA molecules. Whereas homologous recombination is frequently observed during mitosis and meiosis, homeologous recombination is rarely or never seen.

From a breeder's perspective, the limits within which homologous recombination occurs, therefore, define a genetic barrier between species, varieties or lines, in contrast to homeologous recombination which can break this barrier. Homeologous recombination is thus of great importance for plant breeding. Accordingly there is a need for a process for enhancing the frequency of homeologous recombination in plants. In particular, there is a need for a process of increasing homeologous recombination to significantly shorten the length of breeding programs by reducing the number of crosses required to obtain an otherwise rare recombination event.

At least in *Escherichia coli*, homologous and homeologous recombination are known to share a common pathway that requires among others the proteins RecA, RecB, RecC, RecD and makes use of the SOS induced RuvA and RuvB, respectively. It has been suggested that mating induced recombination follows the Double-Strand Break Repair model (Szostak et al., 1983, Cell 33, 25-35), which is widely used to describe genetic recombination in eukaryotes. Following the alignment of homologous or homeologous DNA double helices the RecA protein mediates an exchange of a single DNA strand from the donor helix to the aligned recipient DNA helix. The incoming strand screens the recipient helix for sequence complementarity, seeking to form a heteroduplex by hydrogen bonding the complementary strand. The displaced homologous or homeologous strand of the recipient helix is guided into the donor helix where it base pairs with its counterpart strand to form a second heteroduplex. The resulting branch point then migrates along the aligned chromosomes thereby elongating and thus stabilising the initial heteroduplexes. Single stranded gaps (if present) are closed by DNA synthesis. The strand cross overs (Holliday junction) are eventually resolved enzymatically to yield the recombination products.

Although in wild type *E. coli* homologous and homeologous recombination are thus mechanistically similar if not identical, homologous recombination in conjugational crosses *E. coli* x *E. coli* occurs five orders of magnitude more frequently than homeologous recombination in conjugational crosses *E. coli* x *S. typhimurium* (Matic et al. 1995; Cell 80, 507-515). The imbalance in favour of homologous recombination was shown to be caused largely by the bacterial MisMatch Repair (MMR) system since its

inactivation increased the frequency of homeologous recombination in *E. coli* up to 1000 fold (Rayssiguier et al. 1989, Nature 342, 396-401).

In *E. coli*, the MMR system (reviewed by Modrich 1991, Annual Rev Genetics 25, 229-253) is composed of only three proteins known as MutS, MutL and MutH. MutS recognizes and binds to base pair mismatches. MutL then forms a stable complex with mismatch bound MutS. This protein complex now activates the MutH intrinsic single stranded endonuclease which nicks the strand containing the misplaced base and thereby prepares the template for DNA repair enzymes.

During recombination, MMR components inhibit homeologous recombination. In vitro experiments demonstrated that MutS in complex with MutL binds to mismatches at the recombination branch point and physically blocks RecA mediated strand exchange and heteroduplex formation (Worth et al., 1994; PNAS 91, 3238-3241). Interestingly, the SOS dependent RuvAB mediated branch migration is insensitive to MutS/MutL, explaining the observed slight increase in SOS dependent homeologous recombination. Homeologous mating even induces the SOS response, thereby taking advantage of RuvAB induction (Matic et al. 1995, Cell 80, 507-515).

The MMR system thus appears to be a genetic guardian over genome stability in *E. coli*. In this role it essentially determines the extent to which genetic isolation, that is, speciation, occurs. The diminished sensitivity of the SOS system to MMR, however, allows (within limits) for rapid genomic changes at times of stress, providing the means for fast adaptation to altered environmental conditions and thus contributing to intraspecies genetic variation and species evolution.

The important role of MMR in preserving genomic integrity has been established also in certain eukaryotes. In its efficiency, the human MMR, for example, may even counteract potential gene therapy tools such as triple-helix forming oligonucleotides including RNA-DNA hybrid molecules (Havre et al., 1993, J. Virology 67: 7234-7331; Wang et al., 1995, Mol. Cell. Biol. 15: 1759-1768; Kotani et al., 1996, Mol. Gen. Genetics 250: 626-634; Cole-Strauss et al., 1996, Science 273: 1387-1389). Such oligonucleotides are designed to introduce single base changes into selected DNA target sequences in order to inactivate for example cancer genes or to restore their normal function. The resulting base mismatches however are recognised by the mismatch repair system which then directs removal of the mismatched base, thereby reducing the efficiency of oligonucleotide induced site-specific mutagenesis.

To date, two families of related genes, homologous to the bacterial MutS and MutL genes have been identified or isolated in yeast and mammals (recent reviews by Arnheim and Shibata, 1997, Curr. Opinion Genet. Dev. 7, 364-370; Modrich and Lahue, 1996, Annual Rev. Biochem. 65, 101-133; Umar and Kunkel, 1996, Eur. J. Biochem. 238, 297-307). Biochemical and genetic analysis indicated that eukaryotic MutS homologs (MSH)

and MutL homologs (MLH, PMS), respectively, fulfil similar protein functions as their bacterial counterparts. Their relative abundance, however, could reflect different mismatch specificity and/or specialisation for different tissues or organelles or developmental processes such as mitotic versus meiotic recombination.

5 To date, six different genes homologous to *MutS* have been isolated in yeast (*yMSH*), and their homologs have been found in mouse (*mMSH*) and human (*hMSH*), respectively. Encoded proteins *yMSH2*, *yMSH3* and *yMSH6* appear to be the main *MutS* homologs involved in MMR during mitosis and meiosis in yeast, where the complementary proteins *MSH3* and *MSH6* alternatively associate with *MSH2* to recognise
10 different mismatch substrates (Masischky et al., 1996, *Genes Dev.* 10, 407-420). Similar protein interactions have been demonstrated for the human homologs *hMSH2*, *hMSH3* and *hMSH6* (Acharya et al., 1996, *PNAS* 93, 13629-13634).

MutL homologs (*MLH* and *PMS*), recently reviewed by Modrich and Lahue (1996, *Annual Rev. Biochem.* 65, 101-133) have so far been found in yeast (*yMLH1* and
15 *yPMS1*), mouse (*mPMS2*) and human (*hMLH1*, *hPMS1* and *hPMS2*). The *hPMS2* is a member of a family of at least 7 genes (Horii et al., 1994, *Biochem. Biophys. Res. Commun.* 204, 1257-1264) and its gene product is most closely related to *yPMS1*. Prolla et al. (1994, *Science* 265, 1091-1093) presented evidence for *yPMS1* and *yMLH1* to physically associate with each other and, together, to interact with the *MutS* homolog
20 *yMSH2* to form a ternary complex involved in mismatch substrate binding.

However, while medical interest in mismatch repair has prompted extensive research on MMR in bacteria, yeast and mammals, MMR genes have not been isolated from higher plants prior to the present invention and no attempts to adjust the plant MMR to plant breeding needs have been reported.

25

SUMMARY OF THE INVENTION

According to a first embodiment of the invention, there is provided an isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant. In one form of this embodiment, the invention provides an isolated and purified DNA molecule comprising a
30 polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human. More particularly, the invention provides polynucleotide sequences encoding polypeptides which are homologous to the mismatch repair polypeptides *MSH3* and *MSH6* of *Saccharomyces cerevisiae*. Still more particularly, the invention provides the coding sequences of the genes *AtMSH3* and
35 *AtMSH6* of *Arabidopsis thaliana*, as defined hereinbelow, and polynucleotide sequences encoding polypeptides which are homologous to polypeptides encoded by *AtMSH3* and *AtMSH6*.

According to a second embodiment of the invention, there is provided an isolated and purified polypeptide functionally involved in the DNA mismatch repair system of a plant, for example a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human such as a polypeptide encoded by the genes *AtMSH3* or *AtMSH6* of *Arabidopsis thaliana*, as defined hereinbelow.

According to a third embodiment of the invention, there is provided an isolated and purified DNA molecule comprising a polynucleotide sequence selected from the group consisting of (i) a sequence encoding a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence; and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.

According to a fourth embodiment of the invention there is provided a chimeric gene comprising a DNA sequence selected from the group consisting of (i) a sequence encoding a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence, and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant: together with at least one regulation element capable of functioning in a plant cell. Examples of such regulation elements include constitutive, inducible, tissue type specific and cell type specific promoters such as 35S, NOS, PR1a, AoPR1 and DMC1. Typically, a chimeric gene of the fourth embodiment will also include at least one terminator sequence, more typically exactly one terminator sequence.

In the third and fourth embodiments, said interference, by said polynucleotide sequence, with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair peptide of a yeast or a human typically occurs by hybridisation or by co-suppression.

According to a fifth embodiment of the invention there is provided a plasmid or vector comprising a chimeric gene of the fourth embodiment. A vector of the fifth embodiment may be, for example, a viral vector or a bacterial vector.

According to a sixth embodiment of the invention, there is provided a plant cell stably transformed, transfected or electroporated with a plasmid or vector of the fifth embodiment.

According to seventh embodiment of the invention, there is provided a plant comprising a cell of the sixth embodiment.

According to an eighth embodiment of the invention, there is provided a process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising

transforming or transfecting said plant cell with a DNA sequence of the third embodiment or a chimeric gene of the fourth embodiment or a plasmid or vector of the fifth embodiment, and causing said DNA sequence to express said polynucleotide or said polypeptide.

5 According to a ninth embodiment of the invention, there is provided a process for increasing genetic variation in a plant comprising obtaining a hybrid plant from a first plant and a second plant, or cells thereof, said first and second plants being genetically different; altering the mismatch repair system in said hybrid plant; permitting said hybrid plant to self-fertilise and produce offspring plants; and screening said offspring plants for
10 plants in which homeologous recombination has occurred. For example, homeologous recombination may be evidenced by new genetic linkage of a desired characteristic trait or of a gene which contributes to a desired characteristic trait.

According to a tenth embodiment of the invention there is provided a process for obtaining a plant having a desired characteristic, comprising altering the mismatch repair
15 system in a plant, cell or plurality of cells of a plant which does not have said desired characteristic, permitting mutations to persist in said cells to produce mutated plant cells, deriving plants from said mutated plant cells, and screening said plants for a plant having said desired characteristic.

In a preferred form of the ninth and tenth embodiments of the invention, the step of
20 altering the mismatch repair system comprises introducing into said hybrid plant, plant, cell or cells a chimeric gene of the fourth embodiment and permitting the chimeric gene to express a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence in a mismatch repair gene of the hybrid plant, plant, cell or cells, or a polypeptide capable of disrupting the DNA mismatch repair system of the hybrid
25 plant or cells.

In other embodiments, the invention provides (a) an oligonucleotide capable of hybridising at 45°C under standard PCR conditions to a DNA molecule of the first embodiment; (b) an oligonucleotide capable of hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO: 18 and (c) an oligonucleotide capable of
30 hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO:30; with the proviso that the oligonucleotide of (a), (b) and (c) is other than SEQ ID NO:1 or SEQ ID NO:2.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a diagrammatic representation of the primer sequences used to
35 isolate *AtMSH3*.

Figure 2 is a plasmid map of clone 52, showing restriction enzyme cleavage sites in the 5' half of the full-length cDNA for *AtMSH3*.

Figure 3 is a plasmid map of clone 13, showing restriction enzyme cleavage sites in the 3' half of the full-length cDNA for *AtMSH3*.

Figure 4 is a sequence listing of the coding sequence of *AtMSH3*, together with a deduced sequence of the encoded polypeptide.

5 Figure 5 is a protein alignment of yeast (*Saccharomyces cerevisiae*) and *Arabidopsis thaliana* MSH3 protein.

Figure 6 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH6*.

10 Figure 7 is a plasmid map of clone 43, showing restriction enzyme cleavage sites in the 5' half of the full-length cDNA for *AtMSH6*.

Figure 8 is a plasmid map of clone 62, showing restriction enzyme cleavage sites in the 3' half of the full-length cDNA for *AtMSH6*.

Figure 9 is a sequence listing of the coding sequence of *AtMSH6*, together with a deduced sequence of the encoded polypeptide.

15 Figure 10 is a protein alignment of yeast (*Saccharomyces cerevisiae*) and *Arabidopsis thaliana* MSH6 protein.

Figure 11 is a genomic sequence listing of *AtMSH6*.

Figure 12 is a plasmid map of plasmid pPF13.

Figure 13 is a plasmid map of plasmid pPF14.

20 Figure 14 is a plasmid map of plasmid pCW186.

Figure 15 is a plasmid map of plasmid pCW187.

Figure 16 is a plasmid map of plasmid pPF66.

Figure 17 is a plasmid map of plasmid pPF57.

Figure 18 is a diagrammatic representation of an antisense gene construction for use
25 in homeologous meiotic recombination.

Figure 19 is a plasmid map of plasmid p3243.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the inventors' discovery that there exist in higher plants genes which are homologous to MMR genes in *E. coli*, and to MMR genes in
30 yeasts and humans.

Thus, the inventors have identified genes, herein designated *AtMSH3* and *AtMSH6*, of the plant *Arabidopsis thaliana* which encode the proteins AtMSH3 and AtMSH6. These plant proteins are homologous to yMSH3 and yMSH6, respectively. The present inventors have isolated cDNAs encoding the proteins AtMSH3 and AtMSH6 and have
35 isolated the complete gene encoding AtMSH6. Given the teaching herein, other genes (for example AtMSH2, and genes of other plants) may be obtained which are involved in DNA mismatch repair in plants, including other genes which encode polypeptides homologous to MMR proteins of yeasts or humans, such as genes which encode

polypeptides homologous to yeast MSH2, MLH1 or PMS2, or to human MLH1, PMS1 or PMS2. For example, given the teaching herein, genes of members of the *Brassicaceae* family or of other unrelated families, for example the *Poaceae*, the *Solanaceae*, the *Asteraceae*, the *Malvaceae*, the *Fabaceae*, the *Linaceae*, the *Canabinaceae*, the *Dauaceae* and the *Cucurbitaceae* family, and which encode polypeptides homologous to MMR proteins of yeasts or humans may be obtained.

Examples of plants whose genes encoding polypeptides homologous to MMR proteins of yeasts or humans may be obtained given the teaching herein include maize, wheat, oats, barley, rice, tomato, potato, tobacco, capsicum, sunflower, lettuce, artichoke, safflower, cotton, okra, beans of many kinds including soybean, peas, melon, squash, cucumber, oilseed rape, broccoli, cauliflower, cabbage, flax, hemp, hops and carrot.

Within the meaning of the present invention, a first polypeptide is defined as homologous to a second polypeptide if the amino acid sequence of the first polypeptide exhibits a similarity of at least 50% on the polypeptide level to the amino acid sequence of the second polypeptide.

A procedure which may be followed to obtain genes *AtMSH3* and *AtMSH6* is described in Example 1. Essentially the same technique may be applied to obtain other mismatch repair genes of *Arabidopsis thaliana*, and essentially the same technique as exemplified herein may be applied to cDNA obtained by reverse transcription of RNA from other plants. Alternatively, given the sequence information disclosed herein, other degenerate oligonucleotide primers, especially oligonucleotides of the invention which are capable of hybridising at 45°C under standard PCR conditions (such as the conditions described in Example 1 using primers UPMU and DOMU) to *AtMSH3* and/or *AtMSH6* may be designed and obtained for use in isolating sequences of plant mismatch repair genes which are homologous to *AtMSH3* or *AtMSH6*, from other plants. Similarly, oligonucleotides of the invention which are capable of hybridising at 45°C under standard PCR conditions to plant mismatch repair genes of plants other than *Arabidopsis thaliana* also fall within the scope of the present invention and may be utilised to obtain mismatch repair genes of still other plants. Typically, such oligonucleotides are capable of hybridising at 45°C under standard PCR conditions to a DNA molecule which encodes a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or a human. The temperature at which oligonucleotides of the invention hybridise to *AtMSH3* and/or *AtMSH6*, or to plant mismatch repair genes of plants other than *Arabidopsis thaliana*, or to DNA molecules which encode polypeptides which are homologous to a mismatch repair polypeptide of a yeast or a human may be higher than 45°C, for example at least 50°C, or at least 55°C, or at least 60°C or as high as 65°C.

The successful gene isolation disclosed herein demonstrates for the first time the existence of MMR in higher plants and indicates the presence of other plant MMR genes. For example, genes encoding the plant homologs of MSH1, MSH2, MSH4, MSH5, PMS1, PMS2 and MLH1 may be identified given the teaching herein. Such genes, as well as those specifically described herein, separately or in combination, are useful in manipulating the plant MMR for plant breeding purposes. Thus, for example, the plant MMR may be altered by including in a plant cell a polynucleotide sequence as defined herein above with reference to the third embodiment of the invention, and causing the polynucleotide sequence to express either a polynucleotide which disables a plant MMR gene, or a polypeptide which disrupts the plant's MMR system.

The DNA molecule of the third embodiment of the invention includes a polynucleotide sequence (herein referred to as a MMR altering gene) which may for example encode sense, antisense or ribozyme molecules characterised by sufficient base sequence similarity or complementarity to the gene to be altered to permit the antisense or ribozyme molecule to hybridise with the plant MMR gene in vivo or to permit the sense molecule to participate in co-suppression. Alternatively, the MMR altering gene may encode a protein or proteins which interfere with the activity of a plant MMR protein and thus disrupt the plant's MMR system. For example, such encoded proteins may be antibodies or other proteins capable of interfering with MMR protein function, such as by complexing with a protein functionally involved in plant MMR thereby disrupting the MMR of the plant. An example of such a protein is the MSH3 protein of *Arabidopsis thaliana* described herein or a protein of another plant which is homologous to the MSH3 protein of *A. thaliana*. For instance, overexpression of MSH3 in a plant cell causes MSH2 present in the cell to be substantially completely complexed, disrupting the mismatch repair mechanism or mechanisms in the cell which are functionally dependent on the presence of a complex of MSH2 with MSH6. Similarly, mismatch repair mechanisms which depend on the presence of a complex of MSH2 and MSH3 may be disrupted by the overexpression of MSH6.

A chimeric gene of the fourth embodiment, incorporating a MMR altering gene, may be prepared by methods which are known in the art. Similarly, the MMR altering gene may be introduced into a plant cell, regenerating tissue or whole plant by techniques known in the art as being suitable for plant transformation, or by crossing. Known transformation techniques include *Agrobacterium tumefaciens* or *A. rhizogenes* mediated gene transfer, ballistic and chemical methods, and electroporation of protoplasts.

The MMR altering gene or genes are typically expressed from suitable promoters. Suitable promoters may direct constitutive expression, such as the 35S or the NOS promoter. Usually, however, the promoter will direct either inducible or tissue specific (e.g. callus; embryonic tissue; etc.), cell type specific (e.g. protoplasts; meiocytes; etc.) or developmental (e.g. embryo) expression of the altering gene or genes, in order for the

MMR system to function in tissue types or cell types, or at developmental stages of the plant, in which it is not desirable for the MMR system to be altered. Using such promoters, therefore, the activity of a MMR altering gene may be limited to a specific stage during plant development or it may be altered by controlling conditions external to the plant, and the deleterious effects of a permanently disabled or altered DNA mismatch repair system in a plant may be avoided. Examples of suitable promoters which are not constitutive are known in the art and include inducible promoters such as *PR1a* (reviewed by Gatz, 1997, Annual Rev. Plant Phys. Plant Mol. Biol. 48: 89), tissue specific promoters such as *AoPR1* (Sabahattin et al., 1993, Biotechnology 11: 218), and cell-type specific promoters such as *DMC1*.

A chimeric gene in accordance with the invention may further be physically linked to one or more selection markers such as genes which confer phenotypic traits such as herbicide resistance, antibiotic resistance or disease resistance, or which confer some other recognisable trait such as male sterility, male fertility, grain size, colour, growth rate, flowering time, ripening time, etc.

The process of the tenth embodiment of the invention provides, for example, a process for generating intraspecies genetic variation by altering the mismatch repair system in a plant cell, in regenerating plant tissue or in a whole plant. The plant cell, regenerating tissue or whole plant includes and expresses one or more MMR altering genes which are capable of altering mismatch repair in the plant cell, regenerating tissue or whole plant. Alteration of MMR may be achieved, for example, by inactivating the genes encoding plant MSH3 and/or plant MSH6. It is preferred to inactivate the plant MSH3 and MSH6 encoding genes at the same time and in the same plant cell, regenerating tissue or whole plant. Typically in this preferred form of the invention inactivation of either plant MSH3 or MSH6 alone is insufficient to substantially alter the plant's mismatch repair system and only when both MSH3 and MSH6 are inactivated simultaneously is the plant's mismatch repair system sufficiently altered to prevent the MMR system from recognising base pair mismatches, base insertions or deletions as a result of DNA replication errors, DNA damage, or oligonucleotide induced site-specific mutagenesis. However, in some applications of the invention, inactivation of only one gene may also be used to cause genomic instability or increase the efficiency of site-specific mutagenesis.

If desired, the MMR altering gene or genes may later be rendered non-functional or ineffective, or may be removed from the genome of the plant cell, regenerating tissue or whole plant in order to restore mismatch repair in the plant cell, regenerating tissue or whole plant. The MMR altering gene or genes may be inactivated by means of known gene inactivation tools, such as ribozymes, or may be removed from the genome using gene elimination systems known in the art, such as *CRE/LOX*. It is preferred to render two genes, whose gene products combine to incapacitate MMR, ineffective by separating

the altering genes through segregation. Therefore, in a preferred embodiment of the invention a first plant cell or plant is generated in which only plant *MSH3* is incapacitated, and a second plant cell or plant is generated in which only plant *MSH6* is incapacitated. The combination of both genomes, for example by crossing, then produces significant
5 MMR deficiency in those cells or plants which have inherited both altering genes. If the altering genes are expressed from unlinked loci, gene segregation restores MMR activity in the progeny of the cells or plants.

In a process of the ninth embodiment of this invention, homeologous recombination is enhanced between different genomes, chromosomes or genes in plant cells or plants by
10 altering MMR in said plant cells or plants. Such genomes, chromosomes or genes are characterised in that they originate from different plant families, genera, species, subspecies, plant varieties or lines. Hybrid plant cells or hybrid plants may be produced by crossing, by cell fusion or by other techniques known in the art. These plant cells or plants are further characterised by expressing one or more genes that are capable of
15 altering mismatch repair in the plant cell or plants.

In the process of the ninth embodiment, the homeologous recombination is typically for the purpose of introducing a desired characteristic in the hybrid plant. In this typical application of the process of the ninth embodiment, and in the process of the tenth embodiment the desired characteristic may be any characteristic which is of value to the
20 plant breeder. Examples of such characteristics are well known in the art and include altered composition or quality of leaf or seed derived storage products (e.g. oil, starch, protein), altered composition or quality of cell walls (e.g. decrease in lignin content), altered growth rate, prolonged flowering, increased plant yield or grain yield, altered plant morphology, resistance to pathogens, tolerance to or improved performance under
25 environmental stresses of various kinds, etc.

In a preferred form of the tenth embodiment, an MMR altering gene is co-introduced along with the homeologous genome, chromosome or gene of another plant cell or plant into an MMR proficient plant cell or MMR proficient plant to produce a hybrid plant cell or hybrid plant in which homeologous recombination can occur.
30 Suitably, the MMR proficient plant cell or MMR proficient plant may also include an MMR altering gene. For example a gene capable of inactivating plant *MSH3* may be co-introduced along with the homeologous genome, chromosome or gene of another plant cell or plant into an MMR proficient plant cell or MMR proficient plant in which *MSH6* is inactivated. A resultant hybrid plant in which homeologous recombination occurs will
35 include both the *MSH3* and *MSH6* altering genes and its MMR system will therefore be inactivated.

In this form of the invention, if hybrid plants are to be produced by crossing, the MMR altering gene preferably originates from the male parent, thus ensuring that the

MMR altering gene is always introduced and is not present in the recipient cell. That is, the MMR of the recipient cell, prior to introduction of the MMR altering gene, is typically proficient. Alternatively, if an MMR altering gene is present in a recipient cell it may be ineffective or inefficient on its own, or it may be linked to an inducible or tissue specific or cell type specific promoter which only renders the MMR altering gene active under limited conditions.

Thus, in a preferred form of the process of the ninth embodiment, the MMR system of the hybrid plant is initially unaltered. In this form of the process, the step of altering the mismatch repair system may comprise introducing into the hybrid plant, or cells thereof, a MMR altering gene, such as by *Agrobacterium tumefaciens* or *A. rhizogenes* mediated gene transfer, ballistic and chemical methods, and electroporation of protoplasts.

The MMR altering gene or genes are typically expressed from suitable promoters, as described above. Preferably, the promoter is transcriptionally active in mitotically and meiotically active tissue and/or cells to ensure MMR alteration after chromosome pairing at mitosis and meiosis, respectively. The preferred timing for MMR alteration is at meiosis, because recombinant genomes, chromosomes or genes are directly transmitted to the progeny. A suitable meiocyte specific promoter is for example the *DMC1* promoter from *Arabidopsis thaliana* ssp. *Ler.* (Klimyuk and Jones, 1997, Plant J. 11, 1-14). However, mitotic homeologous recombination is also a desirable outcome as somatic recombination events can be transmitted to offspring due to the totipotency of plant cells and the lack of predetermined germ cells in plants.

If desired, the MMR altering gene or genes may later be rendered non-functional or ineffective, or may be removed from the hybrid plant or hybrid plant cells, in order to restore mismatch repair in the hybrid plant or hybrid plant cells. The MMR altering gene or genes may be inactivated by means of known gene inactivation tools as described herein above.

EXAMPLES

Example 1. Cloning of the *AtMSH3* and *AtMSH6* coding sequences

Isolation of partial *AtMSH3* and *AtMSH6* consensus sequences

Degenerate oligonucleotides UPMU (SEQ ID NO:1) and DOMU (SEQ ID NO:2)

UPMU CTGGATCCACIGGCCIAA(C/T)ATG

DOMU CTGGATCC(A/G)TA(A/G)TGIGTI(A/G)C(A/G)AA

were used to isolate *AtMSH3* and *AtMSH6* sequences by PCR amplification.

Primers UPMU and DOMU correspond to conserved amino acid sequences of the proteins MutS (*E. coli* and *S. typhimurium*), HexA (*S. pneumoniae*), Rep1 (mouse) and Dc1 (human). The conserved regions to which they are targeted are TGNM for UPMU (amino acid positions 852-856 for *AtMSH6* and 816-820 for *AtMSH3*) FATHY or FVTHY

for DOMU (amino acid positions 964-968 for AtMSH6 and 928-932 for AtMSH3, respectively.) These primers have been used to isolate MSH2 and MSH1 from yeast (Reenan and Kolodner, Genetics 132: 963-973 (1992)) and MSH2 from *Xenopus* and mouse (Varlet et al., Nuc. Acids Res. 22:5723-5728 (1994)).

- 5 Template single strand cDNA was produced by reverse transcription of 2 µg total RNA from a cell suspension culture of *Arabidopsis thaliana* ecotype Columbia (Axelos et al. 1989, Mol. Gen. Genetics 219: 106-112). The PCR reaction was performed under the following conditions in a final volume of 100µl: 0.2mM dNTP, 1µM each primer, 1XPCR buffer, 1u *Taq* DNA polymerase (Appligene) in the presence of template cDNA. PCR
- 10 parameters were 5 minutes at 94°C, followed by 30 cycles of 40 seconds at 95°C, 90 seconds at 45°C, 1 minute at 72°C. The amplification products were cloned into pGEM-T vector (Promega) and sequenced. Two different clones were isolated, S5 (350bp) was homologous to *MSH3*, S8 (327bp) was homologous to *MSH6*. Complete cDNA sequences were then isolated according to the Marathon cDNA amplification kit procedure (Clontech).
- 15 In summary, this procedure involves producing double stranded cDNA by reverse transcription of 2µg polyA⁺ RNA from the cell suspension culture of *Arabidopsis*. Adaptors are ligated on each side of the cDNA. The ligated cDNA is used as a template for 5' and 3' RACE PCR reactions in the presence of primers that are specific for the adaptor on one side (AP1 and AP2), and specific for the targeted gene on the other side. A 5' and a 3'
- 20 fragment that overlap are thus produced for each gene. The complete gene coding sequence can be reconstituted taking advantage of a unique restriction site, if available, in the overlapping region. Specific details of this procedure as it was used to isolate *AtMSH3* and *AtMSH6* coding regions, are as follows.

Isolation of *AtMSH3* complete coding sequence

- 25 From the sequence of clone S5, primer 636 (SEQ ID NO:3) was designed:

636 TGCTAGTGCCTCTTGCAAGCTCAT.

Primer AP1 (SEQ ID NO:4) is complementary to a portion of an adaptor sequence which had been ligated to the 5' and 3' ends of *Arabidopsis* cDNA:

AP1 CCATCCTAATACGACTCACTATAGGGC.

- 30 PCR performed on the ligated cDNA with primers 636 and AP1 for the 5' RACE PCR was followed by a second round of amplification with the nested primers AP2 (SEQ ID NO:5) and S525 (SEQ ID NO:6)

AP2 ACTCACTATAGGGCTCGAGCGGC

S525 AGGTTCTGATTATGTGTGACGCTTTACTTA

- 35 (the latter was also designed to correspond to a part of the sequence of clone S5) and produced a 2720bp DNA fragment. Figure 1 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH3*. Another primer (S51, SEQ ID NO:7)

S51 GGATCGGGTACTGGGTTTTGAGTGTGAGG

was designed closer to the 5' border and permitted the determination of 99bp upstream to the ATG initiation codon. For the 3' RACE PCR, a first PCR reaction was performed with primers AP1 and 635 (SEQ ID NO:8).

635 GCACGTGCTTGATGGTGTTCAC

5 followed by a second round of amplification, using the nested primers AP2 and S523 (SEQ ID NO:9)

S523 TCAGACAGTATCCAGCATGGCAGAAGTA

which produced a DNA fragment of 890bp. Both DNA fragments were subcloned into pGEM-T and sequenced. Since PCR amplification using the Expand Long Template PCR System (Boehringer-Mannheim) produced errors in the sequence, new oligonucleotides were designed to isolate those sequences again by PCR, but with the high fidelity DNA polymerase *Pfu*. PCR with primers 1S5 (SEQ ID NO:10) and S53 (SEQ ID NO:11)

1S5 ATCCCGGGATGGGCAAGCAAAAGCAGCAGACGA

S53 GACAAAGAGCGAAATGAGGCCCTTGG

15 amplified the 1244bp fragment clone 52 (SEQ ID NO:12, cloned into pUC18/*Sma*I). PCR with primers S52 (SEQ ID NO:13) and 2S5 (SEQ ID NO:14)

2S5 ATCCCGGGTCAAAATGAACAAGTTGGTTTGTAGTC

S52 GCCACATCTGACTGTTCAAGCCCTCGC

amplified the 2104bp clone 13 (SEQ ID NO:15, cloned into pUC18/*Sma*I). The complete coding sequence of the *AtMSH3* gene was reconstructed in pUC18 by ligating the 5' half of *AtMSH3* (clone 52) to the 3' half of *AtMSH3* (clone 13) after digesting with *Bam*HI which has a unique cleavage site in the overlapping region of both clones. This manipulation yielded plasmid pPF26. The *Sma*I fragment from pPF26 contains the complete *AtMSH3* coding sequence. The remaining primers referred to in Figure 1 are as follows:

S51 GGATCGGGTACTGGGTTTTGAGTGTGAGG (SEQ ID NO:16)

S525 AGGTTCTGATTATGTGTGACGCTTTACTTA (SEQ ID NO:17)

Figures 2 and 3 provide plasmid maps of clones 52 and 13 respectively, showing restriction enzyme cleavage sites. The complete *AtMSH3* coding sequence (SEQ ID NO:18) is 3246bp long and is shown in Figure 4 together with the deduced sequence (SEQ ID NO:19) of the encoded polypeptide. *AtMSH3* is clearly homologous to the yeast and mouse *MSH3* genes. A sequence alignment of polypeptides encoded by *AtMSH3* and that encoded by *Saccharomyces cerevisiae MSH3* is set out in Figure 5.

Isolation of the *AtMSH6* complete coding sequence and genomic sequences

35 The same procedure allowed isolation of the *AtMSH6* cDNA. Figure 6 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH6*. For the 5' RACE PCR, primers 638 (SEQ ID NO:20) and AP1 (SEQ ID NO:4)

638 TCTCTACCAGGTGACGAAAAACCG

allowed the amplification of a 2889 DNA fragment. Primer S81 (SEQ ID NO:21)

S81 CGTCGCCTTTAGCATCCCCTTCCTTCAC

helped define the 142bp upstream to the ATG initiation codon. On the 3' side, RACE PCR was initially performed with primers S823 (SEQ ID NO:22) and AP1 (SEQ ID NO:4).

S823 GCTTGGCGCATCTAATAGAATCATGACAGG

5 and then with the nested primers 637 (SEQ ID NO:23) and AP2 (SEQ ID NO:5).

637 GACAGCGTCAGTTCTTCAGAATGC

to produce a 774bp DNA fragment. As for *AtMSH3*, those fragments were cloned and sequenced. Re-isolation of the DNA sequence using the high fidelity *Pfu* polymerase and newly designed primers 1S8 (SEQ ID NO:24) and S83 (SEQ ID NO:25) (for the 5' side) led
10 to a 2182 bp DNA fragment identified as clone 43 (SEQ ID NO:26, cloned in pUC18/SmaI), and a 1379bp clone identified as clone 62 (SEQ ID NO:27, also cloned in pUC18/SmaI).

1S8 ATCCCGGGATGCAGCGCCAGAGATCGATTTTGT

2S8 ATCCCGGGTTATTTGGGAACACAGTAAGAGGATT (SEQ ID
15 NO:28)

S82 GCGTTCGATCATCAGCCTCTGTGTTGC (SEQ ID NO:29)

S83 CGCTATCTATGGCTGCTTCGAATTGAG

Figures 7 and 8 provide plasmid maps of clones 43 and 62 respectively, showing restriction enzyme cleavage sites. Clones 43 and 62 were digested by the *XmnI* restriction enzyme for
20 which a unique site is present in their overlapping region and then ligated. The complete *AtMSH6* coding sequence (SEQ ID NO:30) is 3330bp long and is shown in Figure 9 together with the deduced sequence (SEQ ID NO:31) of the encoded polypeptide. *AtMSH6* is clearly homologous to the yeast and mouse *MSH6* genes. A sequence alignment of polypeptides encoded by *AtMSH6* and that encoded by *Saccharomyces cerevisiae MSH6* is
25 set out in Figure 10.

An *AtMSH6* genomic sequence was also isolated from a genomic DNA library constituted after partial *Sau3A*I digestion of DNA from the *Arabidopsis* cell suspension. 8062bp were sequenced that covered the *AtMSH6* gene and show colinearity with the cDNA. 16 introns are found scattered along the gene. The complete genomic sequence
30 (SEQ ID NO:98) is shown in Figure 11.

Example 2. A measure of somatic variation in MMR deficient plants

Constructs

Constructs with antisense *AtMSH3* or antisense *AtMSH6* or both *AtMSH3/AtMSH6* under the control of a single 35S promoter have been inserted into the binary vector
35 pPZP121 (Hajdukiewicz et al., Plant Mol. Biol. 23, 793-799) between the right and left borders of the T-DNA. The pPZP121 plasmid confers chloramphenicol resistance to *Escherichia coli* or *Agrobacterium tumefaciens* bacteria. The *aacC1* gene is carried by the T-DNA and allows selection of transformed plant cells on gentamycin (Hajdukiewicz et al., Plant Mol. Biol. 25, 989-994). For the purpose of expressing antisense constructs, a 35S

promoter/terminator cassette with a polylinker was introduced into pPZP121. The 3' ends of the considered genes have been chosen since this region seems more efficient for antisense inhibition. For *AtMSH3* this corresponds to clone 13 (2104bp), for *AtMSH6* this corresponds to clone 62 (1379bp). Clone 13 comprises 2104bp of the 3' region that were cut off the pUC18 vector by *SalI*/*SstI* restriction, blunted with T4 DNA polymerase and ligated into the T4 DNA polymerase blunted *Bam*HI site of pPZP121/35S, creating clone pPF13. The same procedure was followed for the 3' region of *AtMSH6* clone 62 (1379bp) thus creating plasmid pPF14. For the double constructs, the 3' region (from clone 62) of *AtMSH6* was introduced ahead of the *AtMSH3* region into pPF13 creating pCW186 and reciprocally, the 3' region of *AtMSH3* (from clone 13) was introduced ahead of *AtMSH6* into pPF14, creating pCW187.

These constructs were introduced into the Arabidopsis cells (as described below) of wildtype Columbia and of the Columbia tester line.

An alternative strategy to antisense inhibition of *AtMSH6* comes from experiments of Marra et al. (1998. Proc. Natl. Acad. Sci USA 95. 8568-8573) who show that overexpression of functional *MSH3* results in depletion of MSH6 protein in human cells. This depletion may generate a mismatch repair mutant phenotype.

For the purpose of overexpressing functional *AtMSH3* protein in plant cells, the complete *MSH3* coding region was excised from pPF26 (example 1) by digestion with *SmaI*, and was inserted into the *SmaI* site of pCW164. The resulting construct was named pPF66. It contains a complete *AtMSH3* gene under the control of the 35S promoter inside the left (LB) and right (RB) border of the T-DNA. This T-DNA also contains the *hpt2* gene for gentamycin selection. Plasmid pPF66 was introduced into Arabidopsis cells as described below. One cell clone was selected which clearly overexpressed the *AtMSH3* gene as shown by Northern analysis. Figures 12-16 provide plasmid maps of plasmids pPF13, pPF14, pCW186, pCW187 and pPF66, respectively.

Construction of tester construct

For the purpose of Forward Mutagenesis Assays, a tester construct was built containing the coding regions for *nprII*, *codA*, *uidA*. All three genes are driven by the 35S promoter and are terminated by the 35S terminator. This construct was obtained by introducing an *EcoRI* fragment encoding the *codA* cassette (2.5kb) and a *HindIII* fragment encoding the *uidA* (*GUS*) cassette (2.4kb) into the pPZP111 vector (Hajdukiewicz et al., 1994, Plant Mol Biol 23: 793-799) which already contained the *nprII* expression cassette. This new plasmid was named pPF57. *NprII* is used to select for transformed plant cells. *GUS* is used to analyse the degree of gene silencing in the construct (i.e. to identify cell lines in which the transgenes are expressed), and *codA* is used as a marker for forward mutagenesis (described below).

The plasmid map of pPF57 is provided in Figure 17.

Plant cell transformation

The constructs are introduced into *Agrobacterium* by electroporation. Plant cells are then transformed by co-cultivation. A suspension culture of *Arabidopsis thaliana* cells that has been established by Axelos et al. (1992, Plant Physiol. Biochem. 30, 1-6) may be used. One day old freshly subcultured cells are diluted five times into AT medium (Gamborg B5 medium, 30g/l sucrose, 200µg/l NAA). 10µl of saturated *Agrobacterium* containing the transforming T-DNA constructs are added to 10ml diluted cells in a 100ml erlenmeyer. The co-cultivation is agitated slowly (80rpm) for 2 days. The cells are then washed 3 times into AT medium and finally resuspended in the same initial volume (10ml). The culture is agitated for 3 days to allow expression before plating on selection plates (AT/BactoAgar 8g/l+gentamycin 50µg/ml). Transformed individual calli are isolated 3 weeks later.

Tester Strain

The tester construct on plasmid pPF57 was introduced into *Arabidopsis* cells of wildtype Columbia using the transformation protocol described above. Among 10 candidate transformants, one cell clone was shown (by Southern analysis) to have a unique T-DNA insertion. All three genes were shown to be functional in this cell line as indicated by resistance to kanamycin, blue staining in the presence of X-Glu (*GUS*), and sensitivity to 5-fluoro-cytosine (*codA*).

MMR altering genes (described above) were then introduced individually into the tester line and transformed cells are used for analysis of both Microsatellite Instability and Forward Mutagenesis.

Microsatellite analysis

Microsatellites have been described in *Arabidopsis* (Bell and Ecker, 1994, Genomics 19, 137-144). The present Example is based on a study of instability of microsatellites that are polymorphic amongst different ecotypes. DNA is extracted from the transformed calli. Specific primers have been defined that are used to amplify the microsatellite sequence. One of the two primers is previously P³² labelled by T4 kinase. In case of a polymorphic variation, new PCR products appear that do not follow the expected pattern of migration on a polyacrylamide gel. This is a commonly observed feature for MMR deficient cells in yeast or mammalian cells.

In particular, the present Example describes a study on microsatellites ca72 (CA₁₈), nga172 (GA₂₉), and ATHGENEA(A₃₉), chosen because they belong to the types predominantly affected in human mismatch repair deficient tumors. The size of these microsatellites is not conserved from one *Arabidopsis* ecotype to the other.

Arabidopsis cells which are transformed with an MMR altering gene (above) and control cells not expressing the MMR altering gene are allowed to form calli. DNA is

rapidly extracted from the calli and is analysed for microsatellite instability as described in detail by Bell and Ecker 1994, Genomics 19, 137-144. In summary, the relevant microsatellite is amplified by PCR using P32 labelled primers. The PCR products are separated on a DNA sequencing gel for size determination. Size differences between
 5 microsatellites from transformed and control cells not expressing the MMR altering gene in question indicate microsatellite instability as a result of MMR alteration.

The sequences of primers used for PCR amplification of microsatellites ca72 and nga172 are included in Table 1. PCR amplification of microsatellite ATHGENEA made use of a forward primer containing the sequence

10 ACCATGCATAGCTTAACTTCTTG (SEQ ID NO:32)

and of a reverse primer containing the sequence

ACATAACCACAAATAGGGGTGC (SEQ ID NO:33).

The amplification for microsatellite ca72 revealed in Columbia control cells (with respect to the MMR altering gene) a 248 bp long PCR fragment instead of the published
 15 length of 124 bp. DNA sequencing verified this fragment as a CA₁₈ microsatellite.

Forward mutagenesis assay

Tester cells transformed with antisense *AtMSH3* or antisense *AtMSH6* or both *AtMSH3/AtMSH6* are analysed for the stability of the *codA* gene. The functional *codA* gene confers to sensitivity to 5-fluoro-cytosine (5FC), whereas a gene inactivating mutation in
 20 *codA* will confer resistance to 5FC. The frequency of resistant cells is therefore a good indicator of somatic variation as a direct result of MMR alteration. Variants resistant to 5FC are first analysed for GUS activity. If GUS is inactive, 5FC resistance is assumed to be due to gene silencing (all three genes are under the 35S promoter). If GUS is active, 5FC resistance is assumed to be due to forward mutations that have inactivated *codA*. PCR is
 25 then performed on the putative *codA* mutant genes which is then sequenced to confirm the presence of forward mutations in *codA*.

Besides *codA*, other marker genes may also be used for the Forward Mutagenesis Assay such as the *ALS* gene (conferring sensitivity to valine or to sulfonylurea; Hervieu and Vaucheret, 1996, Mol. Gen. Genet. 251 220-224; Mazur et al. 1987, Plant Physiol. 85 1110-
 30 1117).

Example 3. Homeologous meiotic recombination in *Arabidopsis thaliana*

A. Construction of a meiocyte specific gene expression cassette comprising the *DMC1* promoter and the *NOS* terminator

(i) The *DMC1* promoter may be used as published by Klimyuk and Jones, 1997,
 35 Plant J. 11.1-14). To obtain a more convenient alternative for gene cloning, a 3.3 Kb

long subfragment of the *DMC1* promoter was obtained by PCR from genomic DNA of *Arabidopsis thaliana* (ssp. Landsberg erecta "*Ler*").

The PCR was done in three rounds:

Round One: A 3.7 Kb long product was obtained using the forward primer
5 DMCIN-A comprising the sequence

GAAGCGATATTGTTTCGTG (SEQ ID NO:34)

and the reverse primer DMCIN-B comprising the sequence

AGATTGCGAGAACATTCC (SEQ ID NO:35).

The weak amplification product was then used as template for round two and three.

10 Round Two: A 3.1 Kb long product comprising the promoter and the 5' untranslated leader was obtained using forward primer DMCIN-1, which contained the sequence

acgcgtcgacTCAGCTATGAGATTACTCGTG (SEQ ID NO:36)

and introduced a *SalI* cloning site at the 5' end of the promoter fragment, and reverse
15 primer DMCIN-2 which contained the sequence

gctctagaTTTCTCGCTCTAAGACTCTCT (SEQ ID NO:37)

and introduced a *XbaI* site at the 3' end of the PCR fragment.

Round Three: A 0.2 Kb long product comprising the first exon/intron of the *DMC1* promoter was obtained using forward primer DMCIN-3, which contained the sequence

20 gctctagaGCTTCTCTTAAGTAAGTGATTGAT (SEQ ID NO:38)

and introduced a *XbaI* site at the 5' end of the PCR fragment, and reverse primer DMCIN-4, containing the sequence

tccccgggctcgagagatctccatggTTTCTTCAGCTCTATGAATCC (SEQ ID NO:39)

and introduced at the 3' end of the PCR product restriction sites for *NcoI*, *BglII*, *XhoI* and
25 *SmaI*.

The products obtained in round Two and Three were digested with *XbaI* and subsequently ligated to reconstitute a 3.3 Kb long *DMC1* promoter from which the first two in-frame ATG start codons were replaced with a unique restriction site for *XbaI*. This promoter can be cloned between the restriction sites for *SalI* and *SmaI* of p3264, 30 which contains the *SacI-EcoRI* NOS terminator in pBIN19, to yield the entire expression cassette in pBIN19. This cassette contains the following cloning sites: *NcoI*, *BglII*, *XhoI*, *SmaI* and (already present on p3264) *KpnI* and *SacI*.

(ii) Another strategy yielded the following convenient *DMC1* promoter. A 1.8 kb DNA fragment comprising the 3' terminal part of the meiocyte specific *DMC1* promoter
35 was isolated by PCR from purified genomic DNA of *Arabidopsis thaliana* (ssp. Landsberg erecta "*Ler*"). The forward PCR primer (DMC1a) contained the sequence

acgcgtcgacGAATTCGCAAGTGGGG (SEQ ID NO:40)

and introduced a *SalI* cloning site at the 5' end of the promoter fragment. The reverse PCR primer (DMC1b) contained the sequence

tccatggagatctcccggtacCGATTTGCTTCGAGGG (SEQ ID NO:41)

introducing a polylinker region at the 3' end of the promoter fragment. The PCR reaction was carried out with VENT DNA Polymerase (NEB) over 25 cycles using the following cycling protocol: 1 minute at 94°C, 1 minute at 54°C, 2 minutes at 72°C.

5 The PCR reaction yielded a blunt ended DNA fragment which was digested with restriction enzyme *SaII* and was cloned into the cleavage sites of restriction enzymes *SaII* and *SmaI* in plasmid p2030, a pUC118 derivative containing the *SacI-EcoRI* NOS terminator fragment of pBIN121. The cloning yielded plasmid p2031, containing the *DMC1* promoter-polylinker-NOS terminator expression cassette depicted in Figure 18.

10 B. Construction of an *MSH3* antisense gene under the control of the *DMC1* promoter

A 2.1 kb DNA fragment encoding the carboxyterminal part of AtMSH3 was removed from the polylinker of clone 13 described in Example 1 after (i) digestion with *KpnI*, (ii) blunting of the DNA ends generated by *KpnI* and (iii) digestion with *BamHI*. The isolated fragment was then cloned in antisense orientation downstream of the *DMC1*
15 promoter in plasmid p2031, which had been digested with restriction enzymes *SmaI* and *BglII*. This cloning yielded plasmid p2033 (Figure 18).

After digestion of p2033 with *EcoRI*, a 4.1 kb DNA fragment was recovered comprising the *DMC1* promoter, the partial *MSH3* cDNA in antisense orientation with respect to the promoter and the *NOS* terminator. This fragment was cloned into the *EcoRI*
20 restriction site of plant transformation vector pNOS-Hyg-SCV to yield plasmid p3242 (Figure 18).

C. Construction of a combined *MSH6/MSH3* antisense gene under the control of a single *DMC1* promoter

A 3.1 kb fragment, encoding in antisense orientation the partial AtMSH6 and AtMSH3
25 sequences and the 35S terminator, was isolated from pCW186 by digestion with *KpnI*. The fragment was treated with *Klenow* enzyme to blunt both ends. It was then cloned into the *SmaI* site of plasmid p3243 (a pNOS-Hyg-SCV derivative, illustrated in Figure 19), which had been opened to delete the region between the *SmaI* sites. Clones containing the fragment in the antisense orientation with respect to the *DMC1* promoter (described in
30 A(ii) above) were identified by diagnostic digestion with *BamHI*. The selected construct was named p3261.

Another practical way of cloning the double antisense gene is as follows. A 1 kb DNA fragment encoding the carboxyterminal part of AtMSH6 is isolated from clone 62 described in Example 1 after digestion of clone 62 plasmid DNA with *BamHI*, which
35 cleaves in the 5' polylinker region flanking the partial cDNA, and with *EcoRI*, which cleaves within the cDNA. The isolated fragment is treated with *Klenow* enzyme to blunt both its ends and is cloned into the recipient plasmid p2033 or p3242. For the purpose of

cloning, the recipient plasmid may be cleaved with either *AvaI* or *NcoI* and can be blunted with *Klenow* enzyme to produce blunt acceptor ends for fragment cloning. This cloning yields two opposite orientations of cloned fragment DNA with respect to the *DMC1* promoter. These can be identified by diagnostic digestion with restriction enzymes *ScaI* or *XmnI* in conjunction with *SacI*. The selected construct contains the *DMC1* promoter, the combined partial cDNAs for *AtMSH3* and *AtMSH6* (both cloned in antisense orientation with respect to the *DMC1* promoter) and the *NOS* terminator. If the recipient plasmid is p2033, the combined antisense gene under control the single *DMC1* promoter is recovered from the vector after *EcoRI* digestion and is cloned into the *EcoRI* restriction site of pNOS-Hyg-SCV.

D. Construction of a full-length *MSH3* sense gene under control of the *DMC1* promoter for overexpression of functional *MSH3* protein

Overexpression of *MSH3* protein was shown in human cells (Marra et al., 1998, Proc. Natl. Acad. Sci. USA 95, 8568-8573) to complex all available *MSH2* protein. This leaves *MSH6* protein without its partner, leading to the degradation of *MSH6* protein and eventually to a mismatch repair phenotype.

This phenomenon is exploited to increase homeologous meiotic recombination in *Arabidopsis* as an alternative to antisense inhibition of *AtMSH6*. For this purpose the full-length cDNA encoding *AtMSH3* is isolated from plasmid pPF66 by digestion with *SmaI* and is cloned into the *SmaI* site of the *DMC1* expression cassettes described in A(i).

E. Selection of Recombination markers on homeologous chromosomes of *Arabidopsis thaliana* subspecies *Landsberg erecta* (Ler), *Columbia* (Col) and *C24*, respectively

E(i). Visual recombination markers in *Arabidopsis th.* subspecies *C24*:

Agrobacterium mediated transformation with a T-DNA containing a *35S-GUS* gene, inactivated by insertion of a *35S-Ac* transposable element (Finnegan et al., 1993, Plant Mol. Biol. 22, 625-633), had yielded a *C24* line in which the T-DNA construct was integrated into chromosome 2. Genetic and molecular analysis of this line shows that the *Ac* transposon had excised from its T-DNA locus thereby restoring *GUS* activity, but had re-inserted into the chromosome at a distance of 16.4 cM, where it stayed fixed (due to disablement of *Ac*) within the *chlorina* gene. Insertional inactivation of the *chlorina* gene caused a bleached phenotype in those plants that were homozygous for this mutation. Because of the two linked phenotypic markers, *chlorina3A:Ac* and *GUS*, this *C24* line was used in crosses to wildtype Ler for analysis of meiotic homeologous recombination on chromosome 2 in conjunction with molecular recombination markers.

E(ii). Visual recombination markers in *Arabidopsis th.* *Ler*:

The Ler line NW1 (obtained from NASC, Nottingham, UK) contains one recessive visual marker per chromosome. i.e. *an-1* on Chr.1, *py-1* on Chr.2, *gl1-1* on Chr.3, *cer2-1*

on Chr.4. and *msl-1* on Chr.5. This line is used in crosses to wildtype C24 which expresses an MMR altering gene for analysis of meiotic homeologous recombination on chromosomes 1-5 in conjunction with molecular recombination markers listed in Table 1.

Other *Ler* lines from NASC have several visual markers in close proximity to each other on the same chromosome. When these lines are used for hybrid production, analysis of homeologous meiotic recombination may make use entirely of visual recombination markers. Particularly suitable for crossing to C24 wildtype that is expressing a MMR altering gene are the following *Ler* lines:

NW22: relative markers are *dis1* - (4 cM) - *ga4* - (11 cM) - *th1* on chromosome 1.

10 NW10: relevant markers are *tz-201* - (5 cM) - *cer3* on chromosome 5.

NW134, relevant markers are *ug* - (4 cM) - *ga3* on chromosome 5.

NW24 (*abi3-1*) and NW64 (*gll-1*). When present in the same plant on chromosome 3, *abi3-1* and *gll-1* are 13 cM apart. Since this marker combination is not available from NASC, we have combined these markers by crossing line NW24 to line NW64. The F1 offspring were allowed to self-fertilise and to produce F2 seeds. F2 Plants which carry both markers as homozygous traits on the same chromosome can be identified firstly, by germinating F2 seeds on germination medium containing selective concentrations of abscisic acid, and subsequently, by identifying among the abscisic acid resistant plants those individuals which show the glabra phenotype.

20 E(iii) Molecular recombination markers in *Col*, *Ler* and C24:

The genome of *Arabidopsis thaliana* is interspersed with unique base sequences arranged as simple tandem repeats. Allelic repeats can vary in length between different *Arabidopsis* subspecies and when amplified by PCR yield diagnostic DNA products of different length named Simple Sequence Length Polymorphisms (SSLPs). Many SSLPs have been genetically mapped and have been assigned to unique chromosome locations on the recombinant inbred map (Bell and Ecker, 1994, Genomics 19, 137-144; Lister and Deans lines, Weeds World 4i, May 1997).

In Table 1 are listed 28 mapped and established SSLPs between *Ler* and *Col*. A number of PCR primer pairs are described herein (SEQ ID NO:42 to SEQ ID NO:97) which also yielded SSLPs between C24 and *Ler* (19 SSLPs) or between C24 and *Col* (25 SSLPs), respectively. Polymorphic SSLPs can be used as molecular markers in the analysis of homeologous recombination between genomes from these subspecies.

The PCR reactions (25 µL) were carried out over 35 cycles (15 seconds at 94°C, 30 seconds at 55°C and 30 seconds at 72°C), with 0.25 U Taq DNA polymerase and 0.6 µg genomic DNA in reaction buffer containing 2 mM MgCl₂. PCR products were separated by agarose gel electrophoresis (4% ultra high resolution agarose) and visualised by ethidiumbromide staining. The results from the PCR experiments are summarised in

Table 1, which also shows the sequence of PCR primers, primer annealing temperature (T_m), PCR product length and chromosome location of SSLP (with respect to the RI map of May 1997, Weeds World 4i).

F. Production of hybrid plants

- 5 C24 plants heterozygous for *chlorina3A:Ac/GUS* are crossed as male to emasculated wildtype *Ler* to produce *Ler/C24(chlorina3A, GUS)* hybrid seeds.

Due to the heterozygosity of the C24 parent, only 50 % of hybrid plants have inherited the *chlorina3A:Ac/GUS* locus. The remaining 50% of hybrid plants are wildtype with respect to *chlorina3A:Ac/GUS*. Since the mutant locus is linked to a kanamycin
10 resistance gene (contained on the same T-DNA as *GUS*) mutant plants can be pre-selected by germinating hybrid seeds on germination medium containing 50 mg/L kanamycin.

Ler plants homozygous for the five chromosome markers are male sterile (*msl-1*) and are crossed without emasculation to wildtype C24 to produce *Ler(an-1, py-1, gl1-1, cer2-1, msl-1)/C24* hybrid seeds.

- 15 Other *Ler* plants, which are male fertile, are crossed after emasculation of the female parent to produce *Ler/C24* hybrid seeds.

G. Introduction of *MSH3* and *MSH6/3* antisense genes into *Arabidopsis* and analysis of meiotic homeologous recombination

(i) Transformation of hybrid plants and analysis of homeologous meiotic recombination

- 20 The plant transformation vectors comprising the antisense genes described in (B) and (C) above are introduced into *Agrobacterium tumefaciens* strain AGL1 (Lazo et al., 1991, Bio/Technology 9, 963-967) by electroporation. Recombinant *Agrobacterium* clones are selected on LB medium containing 50 mg/L rifampicin and 100 mg/L carbenicillin. Selected clones are used to infect roots of *Arabidopsis* hybrid plants (described in (F)
25 above) using the root transformation protocol of Valvekens et al. (1988, PNAS 85, 5536-5540) except that shoot and root inducing media contain hygromycin (10 mg/L) instead of kanamycin.

Plants regenerated from roots of hybrid plants are genetic clones of root donating plants and therefore are again genetic hybrids of two *Arabidopsis* subspecies described in
30 (F). However, in contrast to the root donating plants, the regenerated hybrid plants also contain the introduced transgene and the co-introduced hygromycin resistance gene with the latter allowing these plants to grow on hygromycin containing culture medium.

Hygromycin resistant plants are then allowed to enter the reproductive phase and to produce gametes by meiotic divisions of microspore and megaspore mothercells. At
35 meiosis, the *DMC1* promoter is activated and can direct the expression of antisense genes described in (B) and (C) above, leading to decreased *MSH6* and/or *MSH3* gene

expression. This in turn depletes the gamete mothercells of MSH6 and/or MSH3 protein, thus causing alteration of MMR during meiotic divisions and increasing the recombination frequency between homeologous chromosomes.

Transgenic plants are then allowed to self-fertilise and to produce seeds. These 5 seeds (F2 seeds with respect to hybrid production), and the plants derived therefrom, carry the homeologous recombination events which can be identified by using the visual and molecular recombination markers described in (E) above.

In case of homeologous recombination between chromosomes of *Ler* and C24(*chlorina3A::Ac. GUS*), the analysis concentrates on chromosome 2 by selecting plants 10 showing the visual phenotypic marker *chlorina*. This marker thus serves as a reference point as it indicates that respective chromosomes 2 originate from C24. Other markers, such as *GUS* or molecular markers, on chromosome 2 may then be used to identify chromosomal regions which are derived from the *Ler* chromosome as a result of homeologous recombination. F2 plants of control transformants not expressing the 15 antisense gene(s) can be analysed in parallel and the results can be used for comparison to homeologous recombination results obtained in antisense plants.

(ii) Transformation of C24 wildtype, hybrid plant production and analysis of homeologous meiotic recombination

Introduction of MMR altering genes into wildtype C24 is done using the root 20 transformation protocol as described in G(i) for transformation of hybrid plants. Transformed plants are selected by resistance to either 10 mg/L hygromycin (in case of transformation with T-DNA's derived from pNOS-Hyg-SCV) or to 50 mg/L kanamycin (in case of transformation with T-DNA's derived from pBIN19).

Transgenic plants are then allowed to self-fertilise and to produce seeds (T1 seeds). 25 Segregation of the antibiotic resistance gene in the T1 population then indicates the number of transgene loci. Lines with a single transgene locus (indicated by a 3:1 ratio of resistant:sensitive plants) are selected and are bred to homozygosity. This is done by collecting selfed seeds (T2) from T1 plants and subsequent testing of at least four independent T2 seed populations for segregation of the antibiotic resistance gene. T2 30 populations which do not segregate the antibiotic resistance gene are assumed to be homozygous for both the resistance gene and the linked MMR altering gene.

C24 plants homozygous for the MMR altering gene are then crossed to *Ler* lines homozygous for recessive visual markers (see E(ii)) to produce C24/*Ler* hybrid plants as described in (F). These F1 hybrids are then allowed to enter the reproductive phase and to 35 produce gametes by meiotic division of microspore and megaspore mothercells. At meiosis, the *DMC 1* promoter is activated and can direct the expression of antisense or sense genes described in (B), (C) and (D) above, leading to decreased *MSH6* and/or *MSH3* gene expression. This in turn depletes the gamete mothercells of *MSH6* and/or *MSH3*

protein, thus causing alteration of MMR during meiotic divisions and increasing the recombination frequency between the homeologous chromosomes of *C24* and *Ler*. Recombination events are then scored in the F2 generation.

For recombination analysis, the hybrid plants are allowed to self-fertilise and to produce F2 seeds. F2 plants are then preselected for a first visual marker. Since this marker is recessive, its visual presence indicates homozygosity for *Ler* DNA at the relevant locus. Those F2 plants which show this first visual marker are then analysed for the presence or absence of a second visual marker which in the *Ler* parent is closely linked to the first marker. Absence of the second visual marker indicates recombination between the relevant *C24* and *Ler* chromosomes between the first and second marker. The frequency of recombination in transgenic hybrids is compared to the recombination frequency in control hybrids not expressing the MMR altering gene.

Examples of recombination analysis are the following.

The *Ler* line NW22(*dis1*, *ga4*, *th1*) is used for crosses to transformed *C24*.

F2 plants are preselected first for thiamine requirement (*th1*) and then are further analysed for re-appearance of wildtype height (loss of *ga4*) and/or re-appearance of normal trichomes (loss of *dis1*) as a result of recombination.

The *Ler* line NW10(*tz-201*, *cer3*) is used for crosses to transformed *C24*.

F2 plants are then preselected first for thiazole requirement (*tz*) and then are further analysed for re-appearance of normal, i.e. non-shiny stems (loss of *cer3*) as a result of recombination.

The *Ler* line NW134 (*ttg*, *ga3*) is used for crosses to transformed *C24*. F2 plants are first preselected for dwarfish appearance (*ga3*) and are then analysed for re-appearance of trichomes (loss of *ttg*) as a result of recombination.

Ler plants homozygous for *abi3-1* and *gll-1* are used for crosses to transformed *C24*. F2 plants are first preselected for their ability to germinate in the presence of abscisic acid and are then analysed for re-appearance of trichomes on the leaves (loss of *gll-1*) as a result of recombination.

In the case of homeologous recombination between transformed *C24* and the *Ler* line NW1 (*an-1*, *py-1*, *gll-1*, *cer2-1*, *msl-1*), recombination analysis is similar the one described above, except that the second marker is not a visual marker but has to be a molecular marker. This is because the *Ler* parent carries only one visual marker per chromosome.

TABLE 1: SSLP Markers in *Arabidopsis thaliana* Subspecies

Chromosome	RI Map Position	PCR Primer Pair	Primer Sequence	T _m [°C]	length/COL	length/LER	length/C24
I	2.3	ATEAT1 F	GCCACTGCGTGAATGATATG	57.8	172	162	162
		ATEAT1 R	CGAACAGCCCAACATTAATTC	58.2			
I	9.3	NGA63 F	AACCAAGGCACAGAAGCG	57.3	111	89	120
		NGA63 R	ACCCAAGTGATCGCCACC	59.6			
I	40.1	NGA248 F	TACCGAACCAAAACACAAAGG	56.1	143	129	no amplific.
		NGA248 R	TCTGTATCTCGGTGAATTCTCC	58.2			
I	81.2	NGA128 F	GGTCTGTTGATGTCGTAAGTCG	60.1	180	190	no amplific.
		NGA128 R	ATCTTGAAACCTTTAGGAGGG	58.2			
I	81.2	NGA280 F	CTGATCTCACGGACAATAGTGC	60.1	105	85	85
		NGA280 R	GGCTCCATAAAAAGTGCACC	57.8			
I	111.4	NGA111 F	CTCCAGTTGGAAGCTAAAGGG	60	128	162	170
		NGA111 R	TGTTTTTTAGGACAAAATGGCG	70			
II	ca. 7.5	NGA168 F	CCTTCACATCCAAAACCCAC	57.8	213	217	208
		NGA168 R	GCACATACCCACAAACCAGAA	57.8			

II	ca. 48	NGA1126L	CGCTACGCTTTTCGGTAAAG	57.8	191	199	196
		NGA1126R	GCACAGTCCAAGTCACAACC	59.9			
II	62.2	NGA361L	AAAGAGATGAGAAATTGGAC	51.7	114	120	114
		NGA361R	ACATATCAATATATTAAGTAGC	49.5			
II	73	NGA168 F	TCGTCTACTGCACTGCCG	59.6	151	135	135
		NGA168 R	GAGGACATGTATAGGAGCCTCG	61.9			
II	ca. 77	AthBIO2 L	TGACCTCCTCTTCCATGGAG	59.9	141	209	139
		AthBIO2 R	TTAACAGAAACCCAAAGCTTTC	54.5			
II	ca. 83	AthUBIQUE L	AGGCAAATGTCCATTTCATTG	54.1	146	148	148
		AthUBIQUE R	ACGACATGGCAGATTCTCTCC	57.8			
III	3.4	NGA172 F	AGCTGCTTCCTTATAGCGTCC	60	162	136	140
		NGA172 R	CATCCGAATGCCATTGTTC	55.4			
III	12.8	NGA126 F	GAAAAACGCTACTTTCGTGG	56.1	119	147	no amplific.
		NGA126 R	CAAGAGCAATATCAAGAGCAGC	58.2			
III	17.5	NGA162 F	CATGCAATTGCACTCTGAGG	55.8	107	89	no amplific.
		NGA162 R	CTCTGTCACCTCTTTCCTCTGG	60.1			

III	81.8	NGA6 F	TGGATTTCCTCCTCTCTTCAC	56.1	143	123	143
		NGA6 R	ATGGAGAAAGCTTACACTGATC	56.1			
IV	19.8	NGA12 F	AATGTTGTCTCTCCCTCCTC	59.9	247	234	220
		NGA12 R	TGATGCTCTCTGAAACAAGAGC	58.2			
IV	24.1	NGA8 F	GAGGGCAAATCTTTATTTCCG	56.1	154	198	190
		NGA8 R	TGGCTTTCGTTTATAAACATCC	54.5			
IV	102	NGA1107 L	GCGAAAAAACAACAAAAATCCA	50.2	150	140	140
		NGA1107 R	CGACGAATCGACAGAATTAGG	58			
V	11.8	NGA225 F	GAAATCCAAATCCAGAGAGG	58	119	189	119
		NGA225 R	TCTCCCCACTAGTTTGTGTCC	60.1			
V	16.7	NGA249 F	TACCGTCAATTTCATCGCC	55.4	125	115	115
		NGA249 R	GGATCCCTAACTGTAAAATCCC	58.2			
V	19.9	CA72 F	AATCCCAGTAACCAACACACA	56.3	124	110	110
		CA72 R	CCCAGTCTAACCCAGACCAC	61.9			
V	20	NGA151 F	GTTTGGGAAGTTTGTCTGG	55.8	150	120	130
		NGA151 R	CAGTCTAAAAGCGAGAGTATGATG	58.6			

V	24	NGA106 F	GTTATGGAGTTCTAGGGCAGG	60.1	157	123	130
		NGA106 R	TGCCCCATTTTGTCTCTC	55.8			
V	37.8	NGA139 F	AGAGCTACCAGATCCGATGG	59.9	174	132	132
		NGA139 R	GGTTTCGTTTCACTATCCAGG	55.8			
V	50	NGA76 F	GGAGAAAATGTCACCTCTCCACC	60.1	231	> 250	300
		NGA76 R	AGGCATGGGAGACATTTACG	57.8			
V	61.1	ATHSO191 L	CTCCACCAATCATGCAAAATG	55.8	148	156	146
		ATHSO191 R	TGATGTTGATGGAGATGGTCA	53.7			
V	81.7	NGA129 F	TCAGGAGGAACATAAGTGAGGG	60.1	177	179	172
		NGA129 R	CACACTGAAGATGGTCTTGAGG	60.1			

CLAIMS

1. An isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant.
- 5 2. A DNA molecule according to claim 1 wherein said polypeptide is homologous to a mismatch repair polypeptide of a yeast or of a human.
3. A DNA molecule according to claim 1 wherein said polypeptide is homologous to AtMSH3 (SEQ ID NO: 19) or to AtMSH6 (SEQ ID NO: 31).
4. An isolated and purified polypeptide functionally involved in the DNA
10 mismatch repair system of a plant.
5. A polypeptide according to claim 4 which is homologous to a mismatch repair polypeptide of a yeast or of a human.
6. An isolated and purified polypeptide selected from the group consisting of a polypeptide encoded by the gene *AtMSH3* (SEQ ID NO: 18), a polypeptide encoded by the
15 gene *AtMSH6* (SEQ ID NO:30), polypeptides homologous to a polypeptide encoded by the gene *AtMSH3* (SEQ ID NO: 18) and polypeptides homologous to a polypeptide encoded by the gene *AtMSH6* (SEQ ID NO:30).
7. An isolated and purified DNA molecule comprising a polynucleotide sequence selected from the group consisting of (i) a sequence encoding a polynucleotide which is
20 capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence; and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.
8. A DNA molecule according to claim 7 comprising a polynucleotide sequence
25 encoding a polynucleotide capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence.
9. A DNA molecule according to claim 8 wherein said polynucleotide is capable
30 of interfering with the expression of a plant polynucleotide sequence is a sense polynucleotide, an antisense polynucleotide or a ribozyme.
10. A DNA molecule according to claim 7 comprising a polynucleotide sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.

11. A DNA molecule according to claim 10 wherein said polypeptide is homologous to AtMSH3 (SEQ ID NO: 19) or to AtMSH6 (SEQ ID NO: 31).

12. A DNA molecule according to claim 10 further comprising a regulation element capable of causing overexpression of said polypeptide in a cell of said plant.

5 13. A chimeric gene comprising:

a DNA sequence selected from the group consisting of (i) a sequence encoding a polynucleotide capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence, and (ii) a
10 sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant; and

at least one regulation element capable of functioning in a plant cell.

14. A chimeric gene according to claim 13 wherein said regulation element is selected from constitutive, inducible, tissue type specific and cell type specific promoters.

15 15. A chimeric gene according to claim 13 comprising a DNA sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant, wherein said regulation element is capable of causing overexpression of said polypeptide in a cell of said plant.

16. A chimeric gene according to claim 13 wherein said regulation element is
20 selected from the group consisting of 35S, NOS, PR1a, AoPR1 and DMC1.

17. A plasmid or vector comprising a chimeric gene according to any one of claims 13-16.

18. A plant cell stably transformed, transfected or electroporated with a plasmid or vector according to claim 17.

25 19. A plant comprising a cell according to claim 18.

20. A plant according to claim 19 selected from plants of the families *Brassicaceae*, *Poaceae*, *Solanaceae*, *Asteraceae*, *Malvaceae*, *Fabaceae*, *Linaceae*, *Canabinaceae*, *Dauaceae* and *Cucurbitaceae*.

21. A process for at least partially inactivating a DNA mismatch repair system of a
30 plant cell, comprising transforming or transfecting said plant cell with a DNA molecule according to any one of claims 1-3 or 7-12 and causing said DNA sequence to express said polynucleotide or said polypeptide.

22. A process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising transforming or transfecting said plant cell with a chimeric gene

according to any one of claims 13-16 and causing said DNA sequence to express said polynucleotide or said polypeptide.

23. A process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising transforming or transfecting said plant cell with a plasmid or vector
5 according to claim 17 and causing said DNA sequence to express said polynucleotide or said polypeptide.

24. A process for increasing genetic variation in a plant comprising obtaining a hybrid plant from a first plant and a second plant, or cells thereof, said first and second plants being genetically different; altering the mismatch repair system in said hybrid plant;
10 permitting said hybrid plant to self-fertilise and produce offspring plants; and screening said offspring plants for plants in which homeologous recombination has occurred.

25. A process according to claim 24 wherein a first gene is incapacitated in said first plant, a second gene is incapacitated in said second plant, and said first and second genes are incapacitated in said hybrid plant thereby altering the mismatch repair system of
15 said hybrid plant.

25. A process according to claim 25 wherein said incapacitation of the mismatch repair system of said hybrid plant is reversible.

26. A process according to claim 24 wherein a new genetic linkage of a desired characteristic trait or of a gene which contributes to a desired characteristic trait is
20 observable in at least one of said offspring plants.

27. A process for obtaining a plant having a desired characteristic, comprising altering the mismatch repair system in a plant, cell or plurality of cells of a plant which does not have said desired characteristic, permitting mutations to persist in said cells to produce mutated plant cells, deriving plants from said mutated plant cells, and screening
25 said plants for a plant having said desired characteristic.

28. A process according to claim 27 wherein said step of altering the mismatch repair system comprises introducing into said hybrid plant, plant, cell or cells a chimeric gene according to claim 13 and permitting the chimeric gene to express a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence in a
30 mismatch repair gene of the hybrid plant, plant, cell or cells, or a polypeptide capable of disrupting the DNA mismatch repair system of the hybrid plant, cell or cells.

29. A process according to claim 28 comprising inactivating an MSH3 gene and/or an MSH6 gene of said plant.

30. A process according to claim 28 comprising inactivating an MSH3 gene and an
35 MSH6 gene of said plant.

31. A process according to claim 27 comprising at least partially inactivating the mismatch repair system of said plant in a predetermined cell type or in a predetermined tissue of said plant.

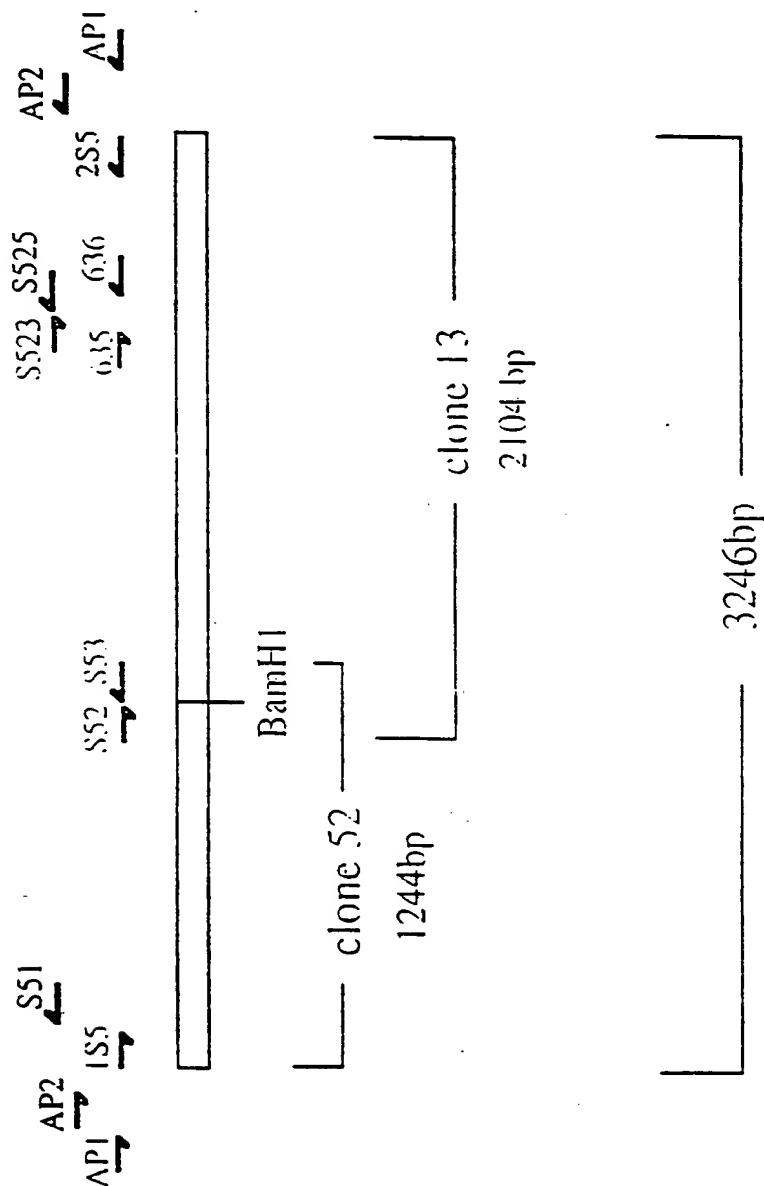
32. A process according to claim 31 further comprising restoring mismatch repair
5 in said cell type or said tissue.

33. An oligonucleotide capable of hybridising at 45°C under standard PCR conditions to a DNA molecule according to claim 1 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

34. An oligonucleotide capable of hybridising at 45°C under standard PCR
10 conditions to the DNA of SEQ ID NO: 18 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

35. An oligonucleotide capable of hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO:30 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

Figure 1



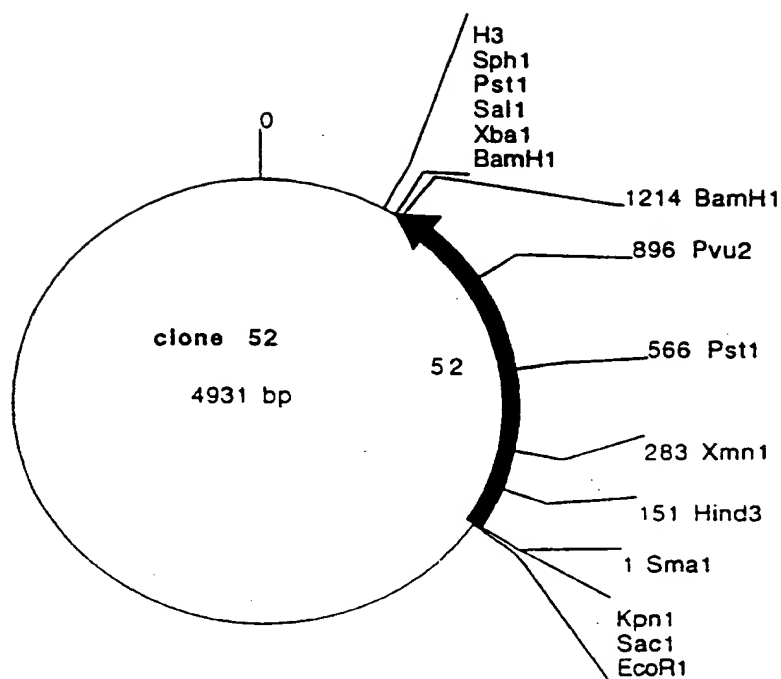
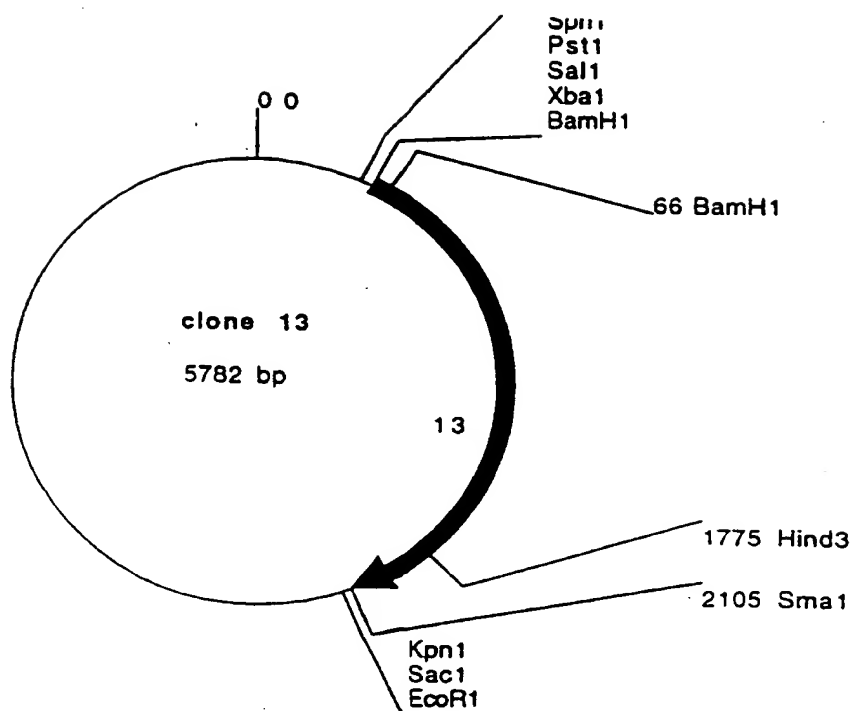


Figure 2

Comments/References: 52= 3' side of S5 (AtMSH3) 1244bp in pUC18/Sma1

**Figure 3**

Comments/References: 13 = 3' side of S5 (AtMSH3) 2104bp in pUC18/SmaI

1	CCTAAGAAAGCGCGGAAATTTGGCAACCCCAAGTTCCCATAGCCACGACGACCTTCCATTCTTCTTAACGGAGGA	80
81	GATTACGAATAAAGCAATT ATG GGC AAG CAA AAG CAG CAG ACG ATT TCT CGT TTC TTC GCT CCC	144
1	M G K Q K Q Q T I S R F A P	15
145	AAA CCC AAA TCC CCG ACT CAC GAA CCG AAT CCG GTA GCC GAA TCA TCA ACA CCG CCA CCG	204
16	K P K S P T H E P N P V A E S S T P P P	35
205	AAG ATA TCC GCC ACT GTA TCC TTC TCT CCT TCC AAG CGT AAG CTT CTC TCC GAC CAC CTC	264
36	K I S A T V S F S P S K R K L L S D H L	55
265	GCC GCC GCG TCA CCC AAA AAG CCT AAA CTT TCT CCT CAC ACT CAA AAC CCA GTA CCC GAT	324
56	A A A S P K K P K L S P H T Q N P V P D	75
325	CCC AAT TTA CAC CAA AGA TTT CTC CAG AGA TTT CTG GAA CCC TCG CCG GAG GAA TAT GTT	384
76	P N L H Q R F L Q R F L E P S P E E Y V	95
385	CCC GAA ACG TCA TCA TCG AGG AAA TAC ACA CCA TTG GAA CAG CAA GTG GTG GAG CTA AAG	444
96	P E T S S S R K Y T P L E Q Q V V E L K	115
445	AGC AAG TAC CCA GAT GTG GTT TTG ATG GTG GAA GTT GGT TAC AGG TAC AGA TTC TTC GGA	504
116	S K Y P D V V L M V E V G Y R Y R F F G	135
505	GAA GAC GCG GAG ATC GCA GCA CGC GTG TTG GGT ATT TAC GCT CAT ATG GAT CAC AAT TTC	564
136	E D A E I A A R V L G I Y A H M D H N F	155
565	ATG ACG GCG AGT GTG CCA ACA TTT CGA TTG AAT TTC CAT GTG AGA AGA CTG GTG AAT GCA	624
156	M T A S V P T F R L N F H V R L V N A	175
625	GGA TAC AAG ATT GGT GTA GTG AAG CAG ACT GAA ACT GCA GCC ATT AAG TCC CAT GGT GCA	684
176	G Y K I G V V K Q T E T A A I K S H G A	195
665	AAC CGG ACC GGC CCT TTT TTC CGG GCA CTG TCG GCG TTG TAT ACC AAA GCC ACG CTT GAA	744
196	N R T G P F F R G L S A L Y T K A T L E	215
745	GCG GCT GAG GAT ATA AGT GGT GGT TGT TGT GGT GAA GAA GGT TTT GGT TCA CAG AGT AAT	804
216	A A E D I S G G C G E E G F G S Q S N	235
805	TTC TTG GTT TGT GTT GTG GAT GAG AGA GTT AAG TCG GAG ACA TTA GGC TGT GGT ATT GAA	864
236	F L V C V V D E R V K S E T L G C G I E	255
865	ATG AGT TTT GAT GTT AGA GTC GGT GTT GGC GTT GAA ATT TCG ACA GGT GAA GTT GTT	924
256	M S F D V R V G V G V E I S T G E V V	275

Figure 4

925	TAT GAA GAG TTC AAT GAT AAT TTC ATG AGA AGT GGA TTA GAG GCT GTG ATT TTG AGC TTG	984
276	Y E E F N D N F M R S G L E A V I L S L	295
985	TCA CCA GCT GAG CTG TTG CTT GGC CAG CCT CTT TCA CAA CAA ACT GAG AAG TTT TTG GTG	1044
296	S P A E L L L G Q P L S Q Q T E K F L V	315
1045	GCA CAT GCT GGA CCT ACC TCA AAC GTT CGA GTG GAA CGT GCC TCA CTG GAT TGT TTC AGC	1104
316	A M A G P T S N V R V E R A S L D C F S	335
1105	AAT GGT AAT GCA GTA GAT GAT GAG GTT ATT TCA TTA TGT GAA AAA ATC AGC GCA GGT AAC TTA	1164
336	N G N A V D E V I S L C E K I S A G N L	355
1165	GAA GAT GAT AAA GAA ATG AAG CTG GAG GCT GCT GAA AAA GGA ATG ATG TCT TGC TTG ACA GTT	1224
356	E D D K E M K L E A A E K G M S C L T V	375
1225	CAT ACA ATT ATG AAC ATG CCA CAT CTG ACT GTT CAA GCC CTC GCC CTA A L T F C H	1284
376	H T I M N M P H L T V Q A L A L T F C H	395
1285	CTC AAA CAG TTT GGA TTT GAA AGG ATC CTT TAC CAA GGG GCC TCA TTT CGC TCT TTG TCA	1344
396	L K Q F G F E R I L Y Q G A S F R S L S	415
1345	AGT AAC ACA GAG ATG ACT CTC TCA GCC AAT ACT CTG CAA CAG TTG GAG GTT GTG AAA AAT	1404
416	S N T E M T L S A N T L Q Q L E V V K N	435
1405	AAT TCA GAT GGA TCG GAA TCT GGC TCC TTA TTC CAT AAT ATG AAT CAC ACA CTT ACA GTA	1464
436	N S D G S E S G S L F H N M N H T L T V	455
1465	TAT GCT TCC AGG CTT CTT AGA CAC TGG GTG ACT CAT CTT CTA TGC GAT AGA AAT TTG ATA	1524
456	Y G S R L L R H W V T H P L C D R N L I	475
1525	TCT GCT CGG CTT GAT GCT GTT TCT GAG ATT TCT GCT TGC ATG GGA TCT CAT AGT TCT TCC	1584
476	S A R L D A V S E I S A C M G S H S S	495
1585	CAG CTC AGC AGT GAG TTG GTT GAA GAA GGT TCT GAG AGA GCA ATT GTA TCA CCT GAG TTT	1644
496	Q L S S E L V E E G S E R A I V S P E F	515
1645	TAT CTC GTG CTC TCC TCA GTC TTG ACA GCT ATG TCT AGA TCA TCT GAT ATT CAA CGT GGA	1704
516	Y L V L S S V L T A M S R S S D I Q R G	535
1705	ATA ACA AGA ATC TTT CAT CGG ACT GCT AAA GCC ACA GAG TTC ATT GCA GTT ATG GAA GCT	1764
536	I T R I F H R T A K A T E F I A V M E A	555
1765	ATT TTA CTT GCG GAG AAG CAA ATT CAG CGG CTT GGC ATA AAG CAA GAC TCT GAA ATG AGG	1824
556	I L L A G K Q I Q R L G I K Q D S E M R	575

Figure 4 (Continued)

1825 576	AGT S	ATG M	CAA Q	TCT S	GCA A	ACT T	GTG V	CGA R	TCT S	ACT T	CTT L	TTG L	AGA R	AAA K	TTG L	ATT I	TCT S	GTT V	ATT I	TCA S
1885 596	TCC S	CCT P	GTT V	GTG V	GTT V	GAC D	AAT N	GCC A	GGA G	AAA K	CTT L	CTC L	TCT S	GCC A	CTA L	AAT N	AAG K	GAA E	GCG A	
1945 616	GTT V	CGA R	GGT G	GAC D	TTG L	CTC L	GAC I	ATA I	CTA L	ATC I	ACT T	TCC S	AGC S	GAC D	CAA Q	TTT F	CCT P	GAG E	CTT A	
2005 636	GAA E	GCT A	CGC R	CAA Q	GCA A	GTT V	TTA L	GTC V	ATC I	AGG R	GAA E	CTG K	GAT L	TCC S	ATA S	GCT I	TCA A	TTT S	F	
2065 656	CGC R	AAG K	AAG K	CTC L	ATT A	CGA I	AAT R	TTG N	GAA L	TTT E	CTT L	CAA Q	GTG V	GGG S	ATC G	ACA I	CAT T	TTG L		
2125 676	ATA I	GAG E	CTG L	CCC P	GTT V	GAT D	TCC S	AAG K	GTC V	CCT P	ATG H	TGG N	GTG W	AAA K	GTA V	AAT N	AGC S	ACC T	AAG K	
2185 696	AAG K	ACT T	ATT I	CGA R	TAT Y	CAT H	CCC P	CCA P	GAA E	ATA I	GTA V	GCT A	GGC G	TTG L	GAT D	GAG E	CTA L	GCA A		
2245 716	ACT T	GAA E	CAT H	CTT L	GCC A	ATT I	GTG V	AAC N	CGA R	GCT A	TGG S	TCG W	GAT D	AGT S	CTC F	AAG L	AGT K	TTC S	AGT S	
2305 736	AGA R	TAC Y	TAC Y	ACA T	GAT D	TTT F	AAG K	GCT A	GCC A	GTT V	CAA Q	GCT A	L	GCA A	CTG L	GAC D	TGT C	TTG L	CAC H	
2365 756	TCC S	CTT L	TCA S	ACT T	CTA L	TCT S	AGA R	AAC N	AAG K	TAT Y	GTC V	CGT R	CCC P	GAG E	TTT F	GTG V	GAT D	GAC C	TGT C	
2425 776	GAA E	CCA P	GTT V	GAG E	ATA I	AAC N	ATA I	CAG Q	TCT S	GGT G	CGT R	CTA H	P	V	CTG L	GAG E	ACT T	ATA I	CAA Q	
2485 796	GAT D	AAC N	TTT F	GTC V	CCA P	AAT N	GAC D	ACA T	ATT I	TTG L	CTA H	CAT A	GCA E	GGA G	TAT Y	TGC C	CAA Q	ATT I	ATC I	
2545 816	ACC T	GGA G	CCT P	AAC N	ATG M	GGA G	AAG K	AGC S	TGC C	Y	ATC I	CGT R	CAA Q	GTT V	ATA L	GCT A	TTT I	TCC S	ATA I	
2605 836	ATG M	GCT A	CAG Q	GTT V	GGT G	TCC S	TTT F	GTA V	CCA P	TCA S	TTC F	GCC A	AAG K	CTG L	CAC H	GTG V	CTT L	GAT D	GGT G	
2665 856	GTT V	TTC F	ACT T	CGG R	ATG M	GGT G	GCT A	TCA S	GAC D	AGT S	ATC I	Q	AGA H	AGT R	ACC S	TTT T	CTA F	GAA L	E	

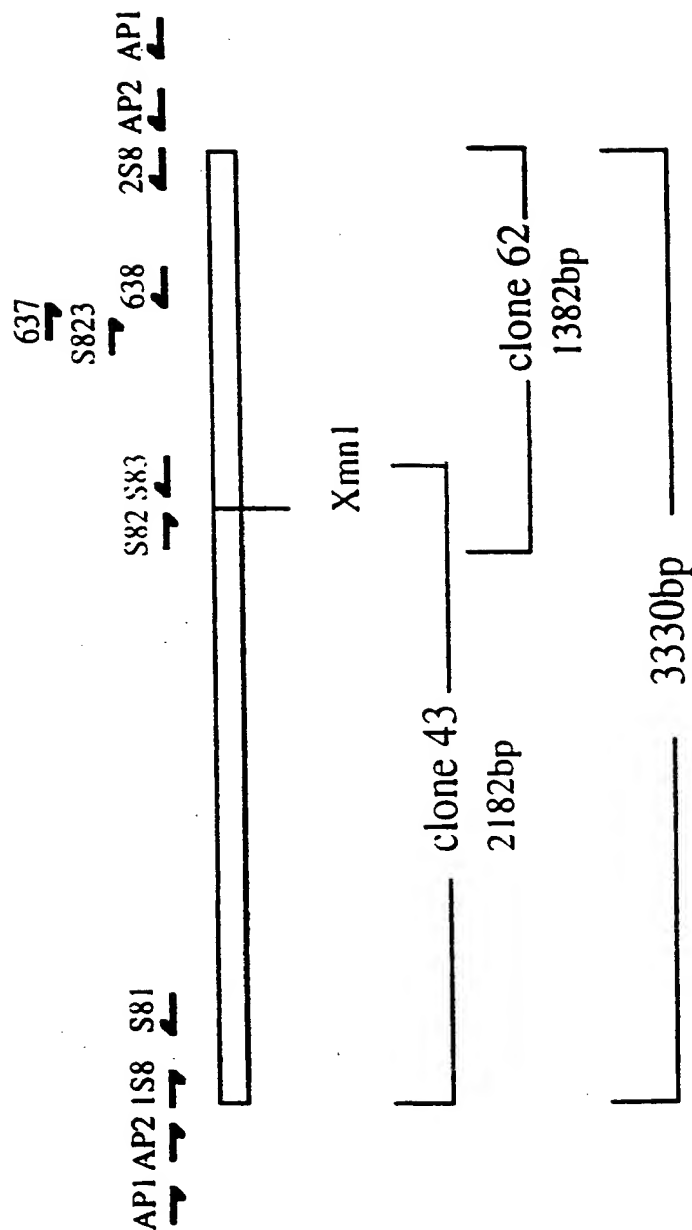
Figur 4 (Continu d)

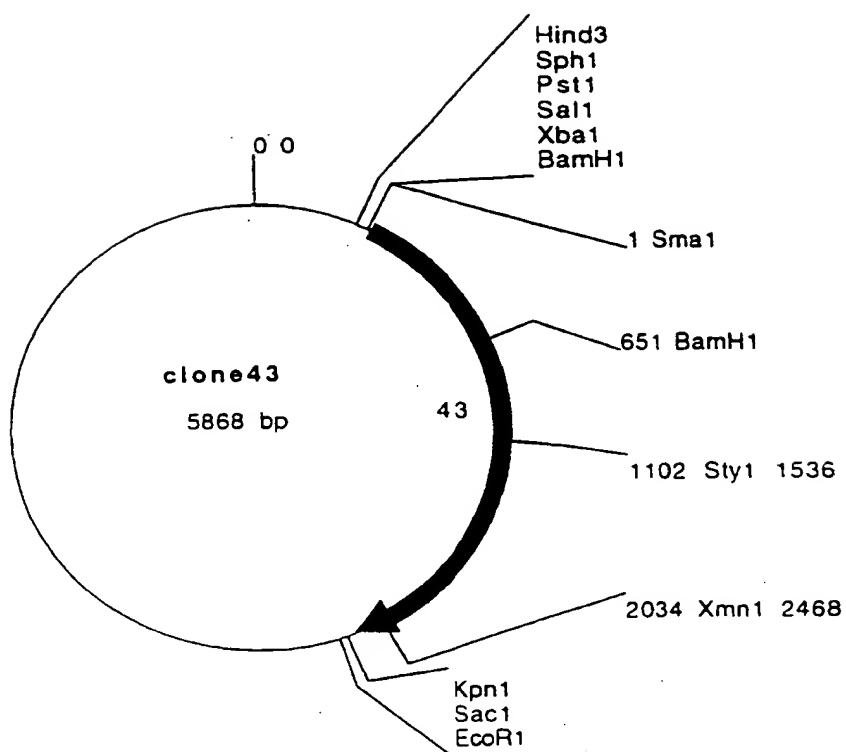
2725 GAA TTA AGT GAA GCG TCA CAC ATA ATC AGA ACC TGT TCT TCT CGT TCG CTT GTT ATA TTA 2784
 876 E L S E A S H I I R T C S S R S L V I L 895
 2785 GAT GAG CTT GGA AGA GGC ACT AGC ACA CAC GAC GGT GTA GCC ATT GCC TAT GCA ACA TTA 2844
 896 D E L G R G T S T H D G V A I A Y A T L 915
 2845 CAG CAT CTC CTA GCA GAA AAG AGA TGT TTG GTT CTT TTT GTC ACG CAT TAC CCT GAA ATA 2904
 916 Q H L L A E K R C L V L F V T H Y P E I 935
 2905 GCT GAG ATC AGT AAC GGA TTC CCA GGT TCT GGT GGG ACA TAC CAT GTC TCG TAT CTG ACA 2964
 936 A E I S N G F P G S V G T Y H V S Y L T 955
 2965 TTG CAG AAG GAT AAA GGC AGT TAT GAT CAT GAT GAT GTG ACC TAC CTA TAT AAG CTT GTG 3024
 956 L Q K D K G S Y D H D V T Y L Y K L V 975
 3025 CGT GGT CTT TGC AGC AGG AGC TTT GGT TTT AAG GTT GCT CAG CTT GCC CAG ATA CCT CCA 3084
 976 R G L C S R S F G F K V A Q L A Q I P P 995
 3085 TCA TGT ATA CGT CGA GCC ATT TCA ATG GCT GCA AAA TTG GAA GCT GAG GTA CGT GCA AGA 3144
 996 S C I R A I S M A A K L E A E V R A R 1015
 3145 GAG AGA AAT ACA CGC ATG GGA GAA CCA GAA CAT GAA GAA CCG AGA GGC GCA GAA GAA 3204
 1016 E R N T R M G E P E G H E E P R G A E E 1035
 3205 TCT ATT TCG GCT CTA GGT GAC TTG TTT GCA GAC CTG AAA TTT GCT CTC TCT GAA GAG GAC 3264
 1036 S I S A L G D L F A D L K F A L S E E D 1055
 3265 CCT TGG AAA GCA TTC GAG TTT TTA AAG CAT GCT TGG AAG ATT GCT GGC AAA ATC AGA CTA 3324
 1056 P W K A F E F L K H A W K I A G K I R L 1075
 3325 AAA CCA ACT TGT TCA TTT TGA TTTAATCTTAACATTATAGCAACI*GCAAGGTCTTGATCATCTGTTAGTTGCG 3397
 1076 K P T C S F * 1082
 3398 TACTAACTT ATG TGT ATT AGT ATA ACA AGA AAA GAG AAT TAG AGAG ATG GAT TCT AAT CCG 3458
 1 M C I S I T R K E N * M D S N P 5
 3459 GTG TTG CAG TAC ATC TTT TCT CCA CCC GCA TAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 3522
 6 V L Q Y I F S P P A * 16

Figure 4 (Continued)

[illegible]

Figure 6



**Figure 7**

Comments/References: 43= 5' side of S8 (AtMSH6) 2182 bp in pUC18/Sma1

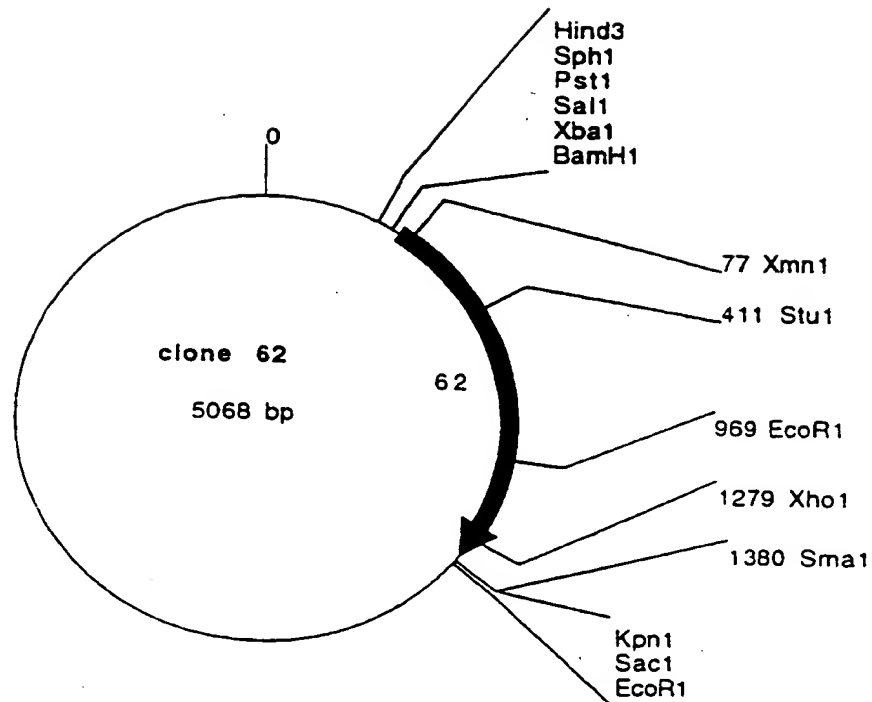


Figure 8

Comments/References: 62= 3' side of S8 (AtMSH6) 1379bp in pUC18/Sma1

1 AAAAGTTGAGCCCTGAGGAGTATCGTTTCGCCCATTTCTACGACGGAAGCGGAAATTTTGGCGCCAATCTTTCCCCCC 80
 81 TTTGGAATTCCTCAGCTCAAAACATCGTTTCTCTCTCACTCTCTCACAATTCACAAAA ATG CAG CGC CAG 153
 1 M Q R Q 4
 154 AGA TCG ATT TTG TCT TTC CAA AAA CCC ACC GCG GCG ACT ACG AAG GGT TTG GTT TCC 213
 5 R S I L S F F Q K P T A A T T K G L V S 24
 214 GGC GAT GCT AGC GGC GGC GGC AGC GGA CCA CGA TTT AAT GTG AAG GAA GGG 273
 25 G D A A S G G G G S G G P R F N V R E G 44
 274 GAT GCT AAA GGC GAC GCT TCT GTA CGT TTT GCT GTT TCG AAA TCT GTC GAT GAG GTT AGA 333
 45 D A K G D A S V R F A V S K S V D E V R 64
 334 GGA ACG GAT ACT CCA CCG GAG AAG GTT CCG CGT CGT GTC CTG CCG TCT GGA TTT AAG CCG 393
 65 G T D T P P E K V P R R V L P S G F K P 84
 394 GCT GAA TCC GCC GST GAT GCT TCG TCC CTG TTC TCC AAT ATT ATG CAT AAG TTT GTA AAA 453
 85 A E S A G D A S S L F S N I M H K F V K 104
 454 GTC GAT GAT CGA GAT TGT TCT GGA GAG AGG AGC CGA GAA GAT GTT GTT CCG CTG AAT GAT 513
 105 V D D R D C S S G E R S R E D V V P L N D 124
 514 TCA TCT CTA TGT ATG AAG GCT AAT GAT GTT ATT CCT CAA TTT CTT TCC AAT AAT GGT AAA 573
 125 S S L C M K A N D V I P Q F R S N N G K 144
 574 ACT CAA GAA AGA AAC CAT GCT TTT AGT TTC AGT GGG AGA GCT GAA CTT AGA TCA GTA GAA 633
 145 T Q E R N H A F S F S G R A E L R S V E 164
 634 GAT ATA GGA GTA GAT GGC GAT GTT CCT GGT CCA GAA ACA CCA GGG ATG CGT CCA CGT GCT 693
 165 D I G V D G D V P G P E T P G M R P A 184
 694 TCT CGC TTG AAG CGA GTT CTG GAG GAT GAA ATG ACT TTT AAG GAG GAT AAG GTT CCT GTA 753
 185 S R L K R V L E D E M T F K E D K V P V 204
 754 TTG GAC TCT AAC AAA AGG CTG AAA ATG CTC CAG GAT CCG GTT TGT GGA GAG AAG AAA GAA 813
 205 L D S N K R L K M L Q D P V C G E K K E 224
 814 GTA AAC GAA GGA ACC AAA TTT GAA TGG CTT GAG TCT TCT CGA ATC AGG GAT GCC AAT AGA 873
 225 V N E G T K F E W L E S S R I R D A N R 244
 874 AGA CGT CCT GAT GAT CCC CTT TAC GAT AGA AAG ACC TTA CAC ATA CCA CCT GAT GTT TTC 933
 245 R R P D D P L Y D R K T L H I P P D V F 264

Figure 9

934 AAG AAA ATG TCT GCA TCA CAA AAG CAA TAT TGG AGT GTT AAG AGT GAA TAT ATG GAC ATT 993
 265 K K M S A S Q K Q Y W S V K S E Y M D I 284
 996 GTG CTT TTC TTT AAA GTG GGG AAA TTT TAT GAG CTG TAT GAG CTA GAT GCG GAA TTA GGT 1053
 285 V L F F K V G K F Y E L Y E L D A E L G 304
 1054 CAC AAG GAG CTT GAC TGG AAG ATG ACC ATG AGT GGT GTG GGA AAA TGC AGA CAG GTT GGT 1113
 305 H K E L D W K M T M S G V G K C R Q V G 324
 1114 ATC TCT GAA AGT GGG ATA GAT GAG GCA GTG CAA AAG CTA TTA GCT CGT GGA TAT AAA GTT 1173
 325 I S E S G I D E A V Q K L L A R G Y K V 344
 1174 GGA CGA ATC GAG CAG CTA GAA ACA TCT GAC CAA GCA AAA GCC AGA GGT GCT AAT ACT ATA 1233
 345 G R I E Q L E T S D Q A K A R G A N T I 364
 1234 ATT CCA AGG AAG CTA GTT CAG GTA TTA ACT CCA TCA ACA GCA AGC GAG GGA AAC ATC GGG 1293
 365 I P R K L V Q V L T P S T A S E G N I G 384
 1294 CCT GAT GCC GTC CAT CTT GCT ATA AAA GAG ATC AAA ATG GAG CTA CAA AAG TGT TCA 1353
 385 P D A V H L L A I K E I K M E L Q K C S 404
 1354 ACT GTG TAT GGA TTT GCT TTT GTT GAC TGT GCT GCC TTG AGG TTT TGG GTT GGG TCC ATC 1413
 405 T V Y G F A F V D C A A L R F W V G S I 424
 1414 AGC GAT GAT GCA TCA TGT GCT CTT GGA GCG TTA TTG ATG CAG GTT TCT CCA AAG GAA 1473
 425 S D D A S C A A L G A L L M Q V S P K E 444
 1474 GTG TTA TAT GAC AGT AAA GGG CTA TCA AGA GAA GCA CAA AAG GCT CTA AGG AAA TAT ACG 1533
 445 V L Y D S K G L S R E A Q K A L R K Y T 464
 1534 TTG ACA GGG TCT ACG GCG GTA CAG TTG GCT CCA GTA CCA CAA GTA ATG GGG GAT ACA GAT 1593
 465 L T G S T A V Q L A P V P Q V M G D T D 484
 1594 GCT GCT GGA GTT AGA AAT ATA ATA GAA TCT AAC GGA TAC TTT AAA GGT TCT TCT GAA TCA 1653
 485 A A G V R N I I E S N G Y F K G S S E S 504
 1654 TGG AAC TGT GCT GTT GAT GGT CTA AAT GAA TGT GAT GTT GCC CTT AGT GCT CTT GGA GAG 1713
 505 W N C A V D G L N E C D V A L S A L G E 524
 1714 CTA ATT AAT CAT CTG TCT AGG CTA AAG CTA GAA GAT GTA CTT AAG CAT GGG GAT ATT TTT 1773
 525 L I N H L S R L K L E D V L K H G D I F 544
 1774 CCA TAC CAA GTT TAC AGG GGT TGT CTC AGA ATT GAT GGC CAG ACG ATG GTA AAT CTT GAG 1833
 545 P Y Q V Y R G C L R I D G Q T M V N L E 564

Figure 9 (C ntinued)

1834	ATA	TTT	AAC	AAT	AGC	TGT	GAT	GGT	GGT	CCT	TCA	GGG	ACC	TTG	TAC	AAA	TAT	CTT	GAT	AAC	1893
565	I	F	N	N	S	C	D	G	G	P	S	G	T	L	Y	K	Y	L	D	N	584
1894	TGT	GTT	AGT	CCA	ACT	GGT	AAG	CGA	CTC	TTA	AGG	AAT	TGG	ATC	TGC	CAT	CCA	CTC	AAA	GAT	1953
585	C	V	S	P	T	G	K	R	L	L	R	N	W	I	C	H	P	L	K	D	604
1954	GTA	GAA	AGC	ATC	AAT	AAA	CGG	CTT	GAT	GTA	GTT	GAA	GAA	TTC	ACG	GCA	AAC	TCA	GAA	AGT	2013
605	V	E	S	I	N	K	R	L	D	V	V	E	E	F	T	A	N	S	E	S	624
2014	ATG	CAA	ATC	ACT	GGC	CAG	TAT	CTC	CAC	AAA	CTT	CCA	GAC	TTA	GAA	AGA	CTG	CTC	GGA	CGC	2073
625	M	Q	I	T	G	Q	Y	L	H	K	L	P	D	L	E	R	L	L	G	R	644
2074	ATC	AAG	TCT	AGC	GTT	CGA	TCA	TCA	GCC	TCT	GTG	TTG	CCT	GCT	CTT	CTG	GGG	AAA	AAA	GTG	2133
645	I	K	S	S	V	R	S	S	A	S	V	L	P	A	L	L	G	K	K	V	664
2134	CTG	AAA	CAA	CGA	GTT	AAA	GCA	TTT	GGG	CAA	ATT	GTG	AAA	GGG	TTG	AGA	AGT	GGA	ATT	GAT	2193
665	L	K	Q	R	V	K	A	F	G	Q	I	V	K	G	F	R	S	G	I	D	684
2194	CTG	TTG	TTG	GCT	CTA	CAG	AAG	GAA	TCA	AAT	ATG	ATG	ATT	TTG	CTT	TAT	AAA	CTC	TGT	AAA	2253
685	L	L	A	L	Q	K	E	S	N	M	S	L	L	L	Y	K	L	C	K		704
2254	CTT	CCT	ATA	TTA	GTA	GGA	AAA	AGC	GGG	CTA	GAG	TTA	TTT	CTT	TCT	CAA	TTT	GAA	GCA	GCC	2313
705	L	P	I	L	V	G	K	S	G	L	E	L	F	L	S	Q	F	E	A	A	724
2314	ATA	GAT	AGC	GAC	TTT	CCA	AAT	TAT	CAG	AAC	CNA	GAT	GTG	ACA	GAT	GAA	AAC	GCT	GAA	ACT	2373
725	I	D	S	D	F	P	N	Y	Q	N	Q	D	V	T	D	E	N	A	E	T	744
2374	CTC	ACA	ATA	CTT	ATC	GAA	CIT	TTT	ATC	GAA	AGA	GCA	ACT	CAA	TGG	TCT	GAG	GTC	ATT	CAC	2433
745	L	T	I	L	I	E	L	F	I	E	R	A	T	Q	W	S	E	V	I	H	764
2434	ACC	ATA	AGC	TGC	CTA	GAT	GTC	CTG	AGA	TCT	TTT	GCA	ATC	GCA	GCA	AGT	CTC	TCT	GCT	GGA	2493
765	T	I	S	C	L	D	V	L	R	S	F	A	I	A	A	S	L	S	A	G	784
2494	AGC	ATG	GCC	AGG	CCT	GTT	ATT	TTT	CCC	GAA	TCA	GAA	GCT	ACA	GAT	CAG	AAT	CAG	AAA	ACA	2553
785	S	M	A	R	P	V	I	F	P	E	S	E	A	T	D	Q	N	Q	K	T	804
2554	AAA	GGG	CCA	ATA	CTT	AAA	ATC	CAA	GGA	CTA	TGG	CAT	CCA	TTT	GCA	GTT	GCA	GCC	GAT	GGT	2613
805	K	G	P	I	L	K	I	Q	G	L	W	H	P	F	A	V	A	A	D	G	824
2614	CAA	TTG	CCT	GTT	CCG	AAT	GAT	ATA	CTC	CTT	GGC	GAG	GCT	AGA	AGA	AGC	AGT	GGC	AGC	ATT	2673
825	Q	L	P	V	P	N	D	I	L	L	G	E	A	R	S	S	S	G	S	I	844
2674	CAT	CCT	CGG	TCA	TTG	TTA	CTG	ACG	GGA	CCA	AAC	ATG	GGC	GGA	AAA	TCA	ACT	CTT	CTT	CGT	2733
845	H	P	R	S	L	L	L	T	G	P	N	M	G	G	K	S	T	L	L	R	864

Figure 9 (Continued)

2734	GCA ACA TGT CTG GCC GTT ATC TTT GCC CAA CTT GGC TGC TAC GTG CCG TGT GAG TCT TGC	2793
865	A T C L A V I F A Q L G C Y V P C E S C	884
2794	GAA ATC TCC CTC GTG GAT ACT ATC TTC ACA AGG CTT GGC GCA TCT GAT AGA ATC ATG ACA	2853
885	E I S L V D T I F T R L G A S D R I M T	904
2854	GGA GAG AGT ACC TTT TTG GTA GAA TGC ACT GAG ACA GCG TCA GTT CTT CAG AAT GCA ACT	2913
905	G E S T F L V E C T E T A S V L Q N A T	924
2914	CAG GAT TCA CTA GTA ATC CTT GAC GAA CTG GGC AGA GGA ACT AGT ACT TTC GAT GGA TAC	2973
925	Q D S L V I L D E L G R G T S T F D G Y	944
2974	GCC ATT GCA TAC TCG GTT TTT CGT CAC CTG GTA GAG AAA GTT CAA TGT CGG ATG CTC TTT	3033
945	A I A Y S V F R H L V E K V Q C R M L F	964
3034	GCA ACA CAT TAC CAC CCT CTC ACC AAG GAA TTC GCG TCT CAC CCA CGT GTC ACC TCG AAA	3093
965	A T H Y H P L T K E F A S H P R V T S K	984
3094	CAC ATG GCT TGC GCA TTC AAA TCA AGA TCT GAT TAT CAA CCA CGT GGT TGT GAT CAA GAC	3153
985	H M A C A F K S R S D Y Q P R G C D Q D	1004
3154	CTA GTG TTC TTG TAC CGT TTA ACC GAG GGA GCT TGT CCT GAG AGC TAC GGA CTT CAA GTG	3213
1005	L V F L Y R L T E G A C P E S Y G L Q V	1024
3214	GCA CTC ATG GCT GGA ATA CCA AAC CAA GTG GTT GAA ACA GCA TCA GGT GCT CAA GCC	3273
1025	A L M A G I P N Q V V E T A S G A A Q A	1044
3274	ATG AAG AGA TCA ATT GGG GGA AAC TTC AAG TCA ACT GAG CTA ACA TCT GAG TTC TCA AGT	3333
1045	M K R S I G E N F K S S E L R S E F S S	1064
3334	CTG CAT GAA GAC TGG CTC AAG TCA TTG GTG GGT ATT TCT CGA GTC GCC CAC AAC AAT GCC	3393
1065	L H E D W L K S L V G I S R V A H N N A	1084
3394	CCC ATT GGC GAA GAT GAC TAC GAC ACT TTG TTT TGC TTA TGG CAT GAG ATC AAA TCC TCT	3453
1085	P I G E D D Y D T L F C L W H E I K S S	1104
3454	TAC TGT GTT CCC AAA TAA ATG GCT ATG ACA TAA CACTATCTGAAGCTCGTTAAGTCTTTTGCCTCTCT	3521
1105	Y C V P K * M A M T	5
3522	G ATG TTT ATT CCT CTT AAA AAA TGC TTA TAT ATC AAA AAA TTG TTT CCT CGA TTA AAA	3579
1	M F I P L K K C L Y I K K L F P R L K	19
3580	AAA AAA AAA AAA AAA AAA AAA AAA	3606
20	K K K K K K K K K K	28

Figure 9 (C ntinued)

Figure 10

1
 1 MAPATPKTBTANTZHOZTSS
 69
 105
 163
 210
 269
 312
 372
 417
 462
 512
 562
 621
 649
 729
 725
 831
 902
 939
 905
 1014
 1010
 1131
 1103
 1235

TTTTTTGGTTGCTAACAAATAAAGGTATACGGTTTTATGTCATCAATATAA	50
CTATATATAAAAGAAATGAAAGATATATATTGTTTTTTCATTTATCAAAC	100
AAAACAACAAGACTTTTTTTTTTACTTTTTTACATTGGTCAACAAAATACAA	150
GATAAACGACATCGTTTAAATCATTTCCTCAATTTTACCCCTAAGTTTAACA	200
CCTAGAACCTTCTCCATCTTCGCAAGCACAGCCTGATTAGGAACAGCTTT	250
ACCATTCTCATATTCTGAACTACCTGAGTCCTCTCATTGATCTGTTTCG	300
CCAAATCCGCTTGTGACATCTTCTTCTCCAATCTCGCTTTCTGTATCATC	350
AACCTCACCTCTGCTTTACACGATCCATCGCCGAGGCTCTGTTTCTTC	400
TTCCAGCTTCTTCGTGTTAATCACCGGAACCGCCGTAGATTTCCCTTTT	450
TGTTCGAACCGGCATCGAATTTCTTAACCGTTTGAACCGCGACACCGTTT	500
CTCAGAGCTGCGTTAACCGCTTTTCGGATCGCGTAGGTCTTGGCTCTTTTG	550
TTTTGATTTGTGGAGAACTACTGGTTCCCAGTCTTGTGTTACTGCTCCTG	600
GGTATCTGCTCGGCATCGTCGATGAATTGAGAGAAAGGAACAACGCGAAA	650
ATTTTATTAATCTGAGTTTGAATTTGAGAAACGATGAAGATGAAGAATG	700
TTGTTGAGAGGATTGTGATATTATATATACGAAGATTGGTTTCTGGAGA	750
ATTGATCATCTTTTCTCCATTTTCGTCTCTGGAACGTTCTTAGAGATG	800
ATTGACGACGTGTCATTATCTGATTTGCAGTTAACCAATGCTTTTTGGGT	850
TGGATTCGTGGTACACCATATTATCCGATTTGGCTCAATGGTTTTATATA	900
AATTTGGTTTTTCGGTTTCGGTTATGAGTTATCATTAAATTAAGCTAACCA	950
AAAATTTTCGTAAAATTTATTTTCGGTTTCAATTCGGATCCCTTACTTCCA	1000
GAACCGAATTATTCGAAACCGGGGTTAGCCGAACCGAATACCAATGCCTG	1050
ATTGACTCGTTGGCTAGAAAGATCCAACGGTATACAATAATAGAACATAA	1100
ATCGGACGGTCATCAAAGCCTCAAAGAGTGAACAGTCAACAAAAAAGTT	1150
GAGCCCTGAGGAGTATCGTTTCCGCCATTTCTACGACGCAAGGCGAAAAT	1200
TTTTGGCGCCAATCTTTCCCCCTTTTGAATTCTCTCAGCTCAAACATC	1250
GTTTCTCTCTCACTCTCTCTCACAAATCCAAAAAATGCAGCGCCAGAGAT	1300
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Figure 11

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Figure 11 (Continued)

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Figure 11 (Continued)

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Figure 11 (Continued)

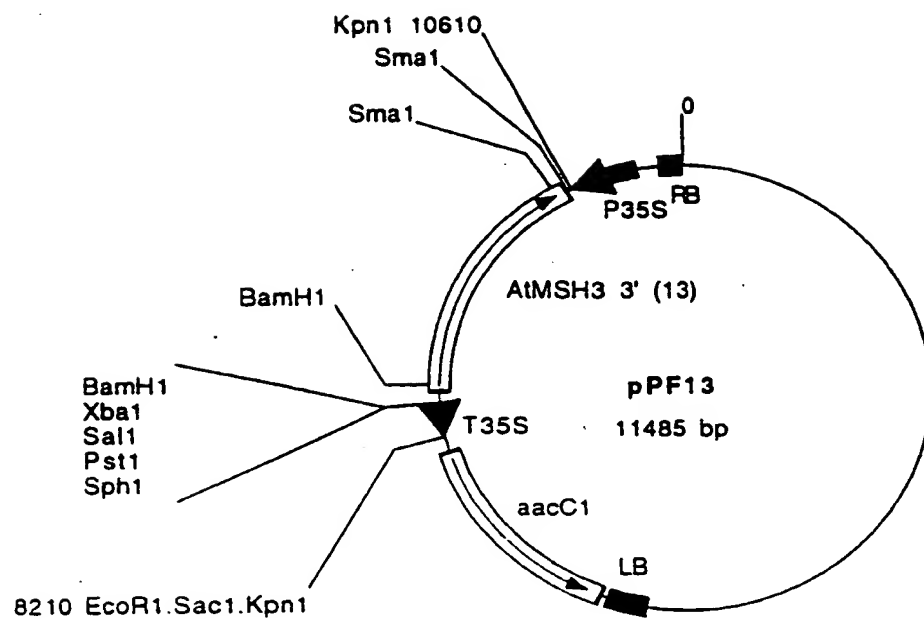


Figure 12

Comments/References: AtMSH3 3' side antisense : AtMSH3 3' (13 = 2104bp) from pUC18/13 Sal1/Sst1/T4 into pCW164 BamH1/T4 in Agrobacterium LBA44O4

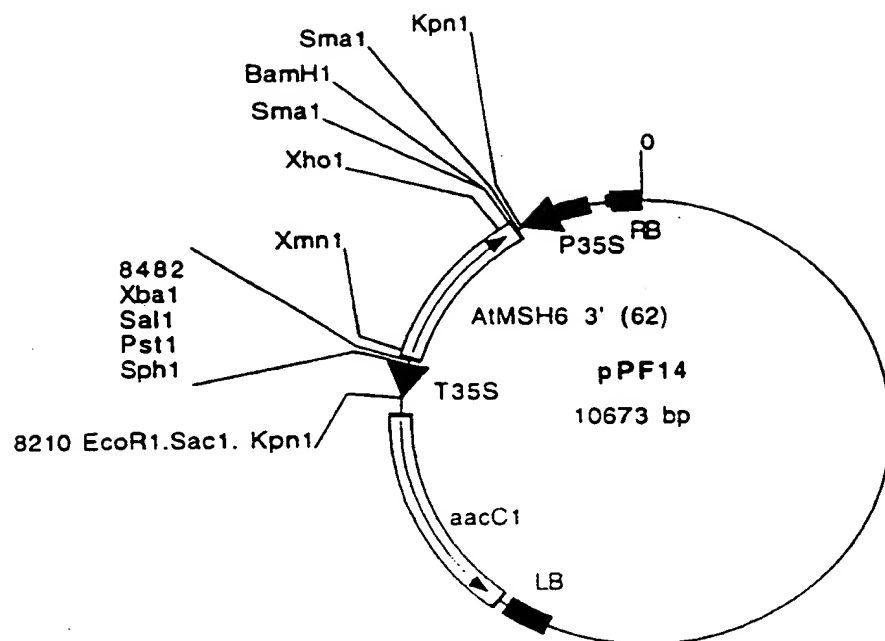


Figure 13

Comments/References: AtMSH6 (S8) 3' side antisens : 62 Sal1/Sst1/T4 (1379bp)
into pCW164 BamH1/T4

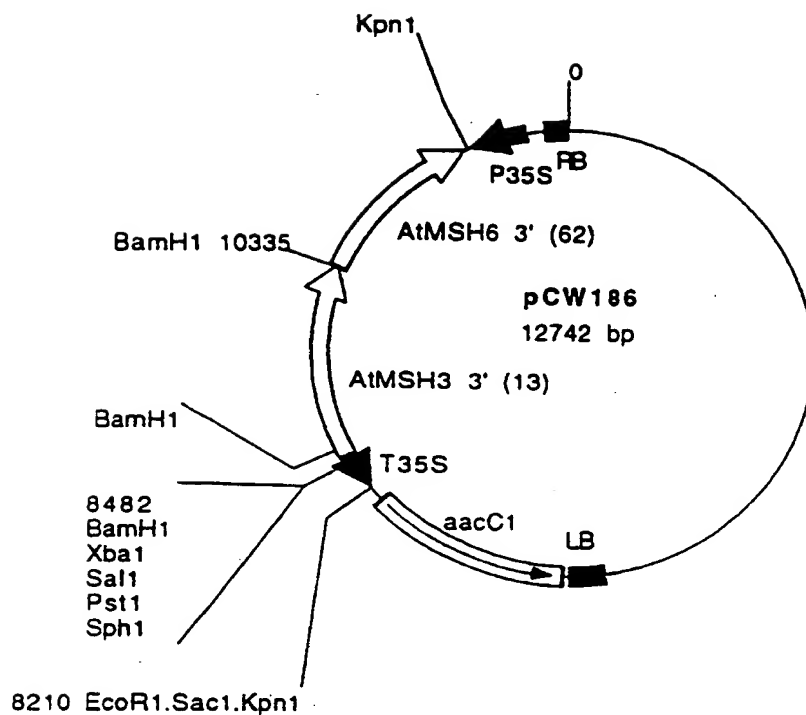


Figure 14

Comments/References: AtMSH6 3'/AtMSH3 3' antisense : AtMSH6 (S8) 3' side (62=1379bp)
Sal1/Sst1/T4 into pPF13 (pCW164 AtMSH3 (S5) 3' side (13=2104) antisense)/Sma1. in
LBA4404

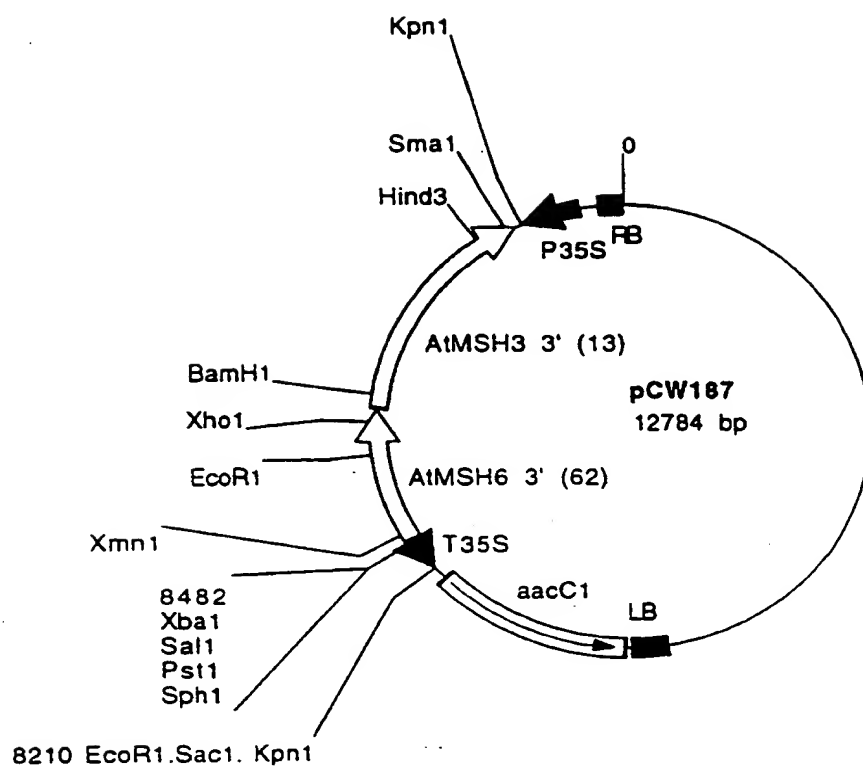


Figure 15

Comments/References: AtMSH3 3'/AtMSH6 3' antisens (D) : AtMSH3 (S5) 3' side (13=2104bp) Sal1/Sst1/T4 into pPF14 (AtMSH6 (S8) 3'side (62=1379bp) antisense into pCW164)/Sma1. in LBA4404

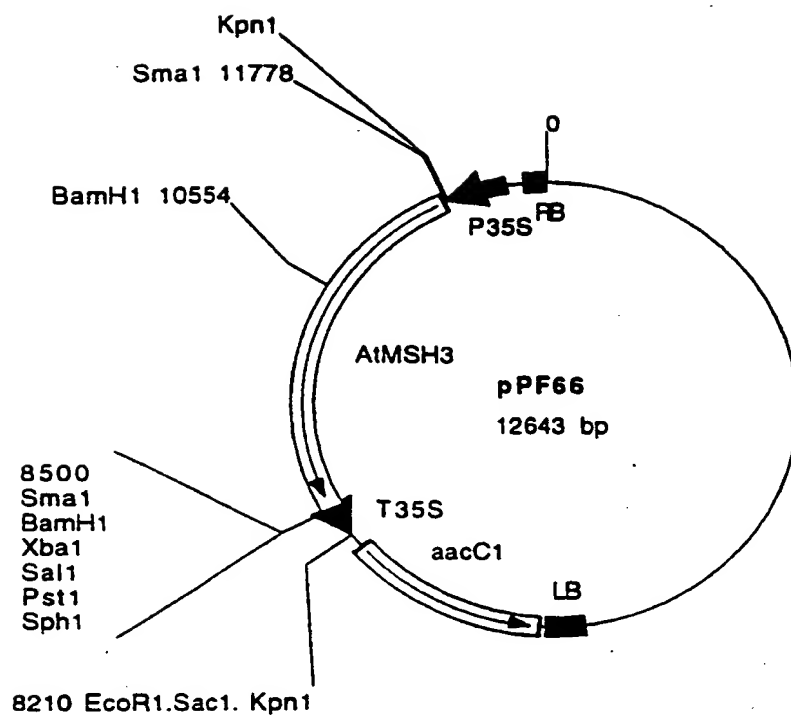


Figure 16

Comments/References: AtMSH3 (S8) complete, sense orientation : pPF26 (3342bp)
Sma1 into pCW164 Sma1

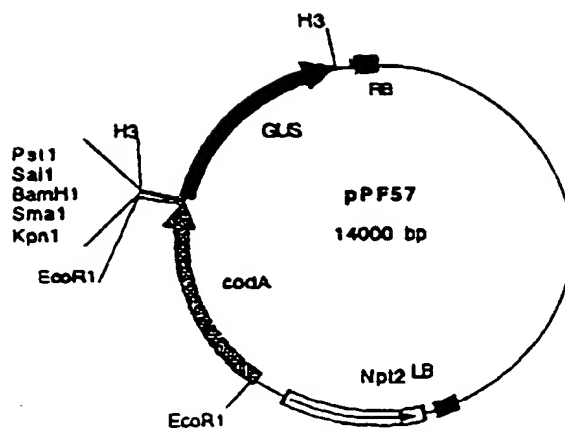


Figure 17

Comments/References: pPZP111 with codA EcoRI cassette in EcoRI site and Hind3 GUS cassette in Hind3 site. KanR. All genes under Promoter/terminator 35S

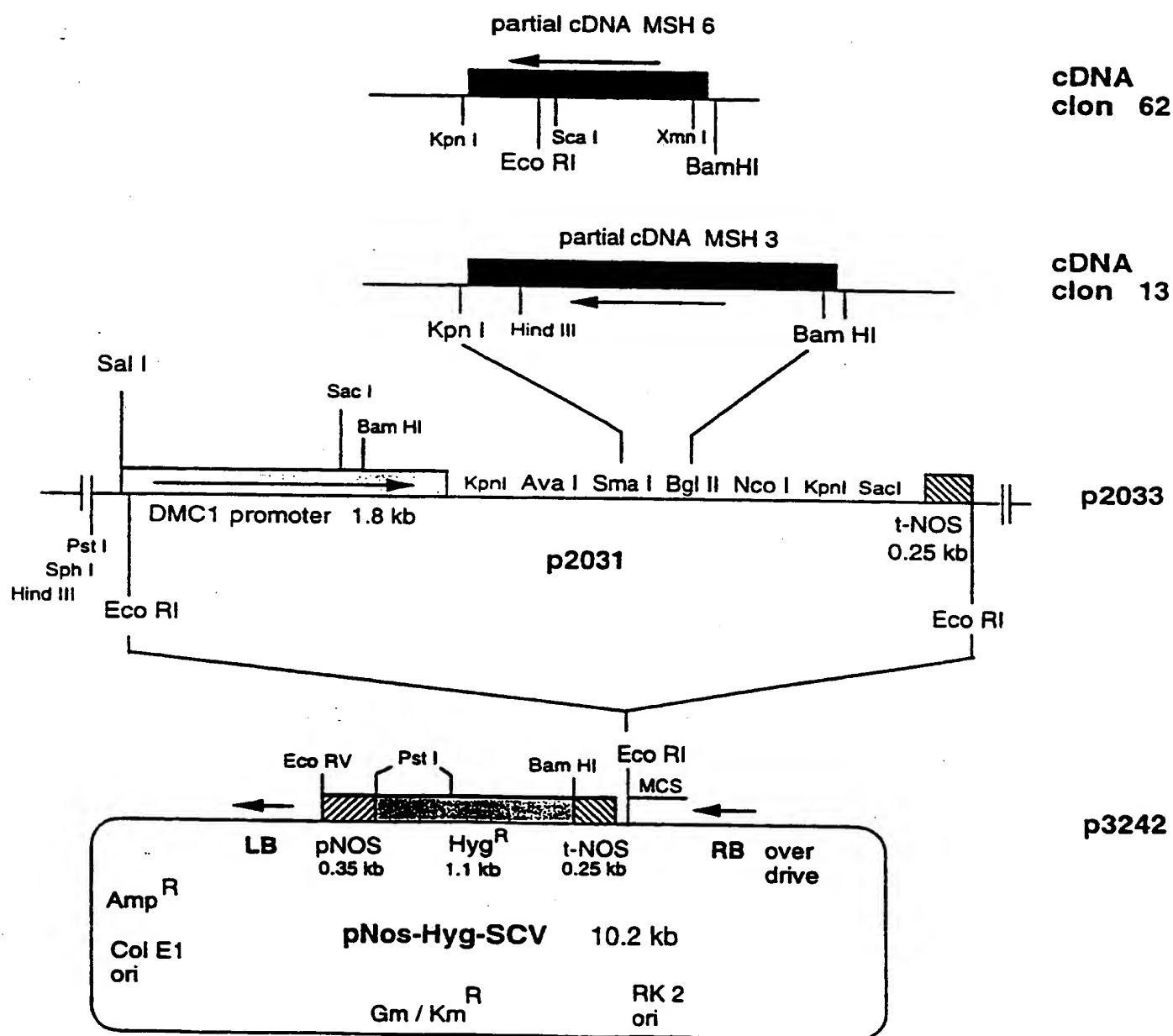
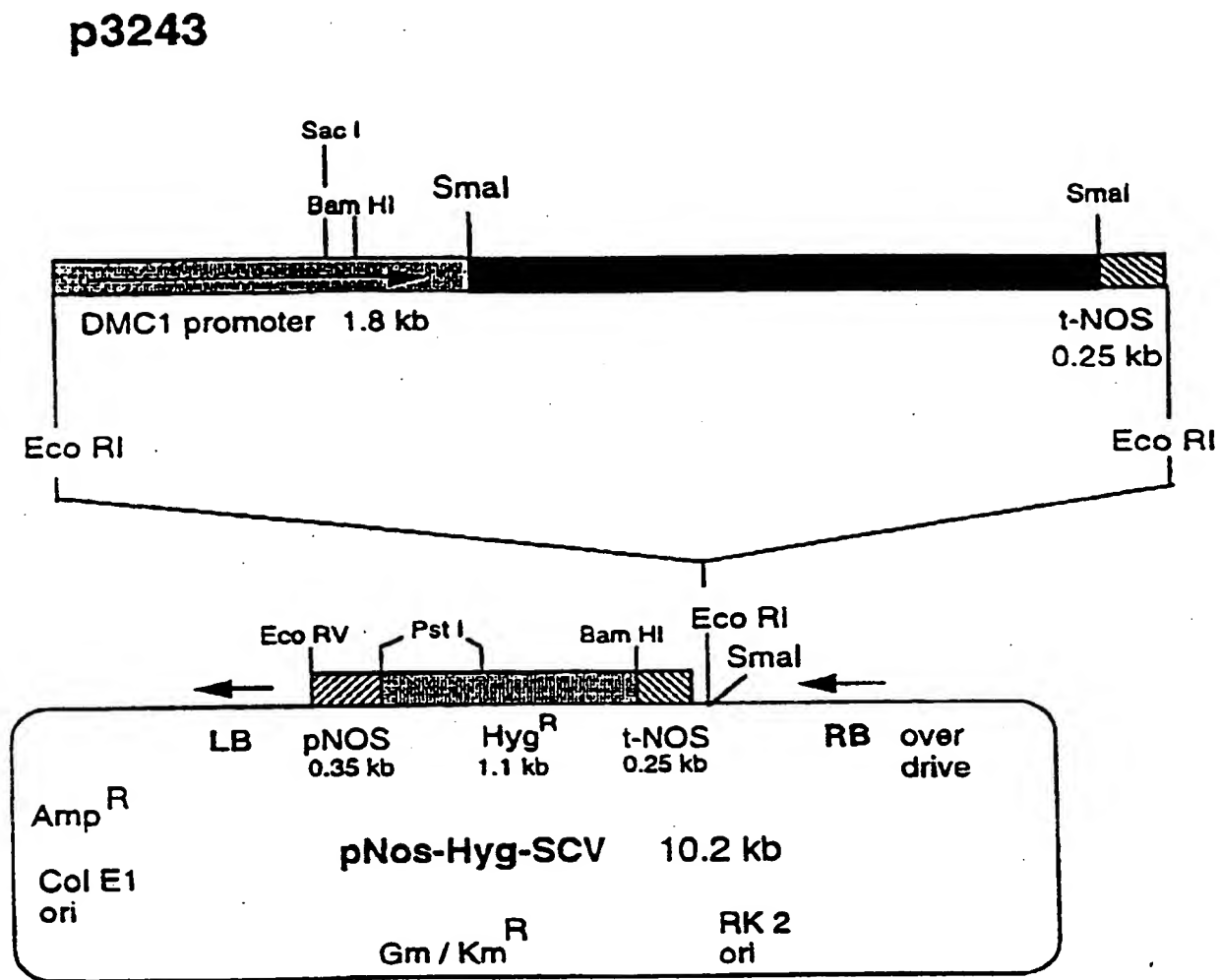
Figure 18

Figure 19

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147

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195

ccg cca ccg aag ata tcc gcc act gta tcc ttc tct cct tcc aag cgt
 Pro Pro Pro Lys Ile Ser Ala Thr Val Ser Phe Ser Pro Ser Lys Arg
 35 40 45

243

aag ctt ctc tcc gac cac ctc gcc gcc gcg tca ccc aaa aag cct aaa
 Lys Leu Leu Ser Asp His Leu Ala Ala Ala Ser Pro Lys Lys Pro Lys
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291

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 Leu Ser Pro His Thr Gln Asn Pro Val Pro Asp Pro Asn Leu His Gln
 65 70 75 80

339

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Arg	Phe	Leu	Gln	Arg	Phe	Leu	Glu	Pro	Ser	Pro	Glu	Glu	Tyr	Val	Pro	
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gaa	acg	tca	tca	tcg	agg	aaa	tac	aca	cca	ttg	gaa	cag	caa	gtg	gtg	435
Glu	Thr	Ser	Ser	Ser	Arg	Lys	Tyr	Thr	Pro	Leu	Glu	Gln	Gln	Val	Val	
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Glu	Leu	Lys	Ser	Lys	Tyr	Pro	Asp	Val	Val	Leu	Met	Val	Glu	Val	Gly	
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Tyr	Arg	Tyr	Arg	Phe	Phe	Gly	Glu	Asp	Ala	Glu	Ile	Ala	Ala	Arg	Val	
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Leu	Gly	Ile	Tyr	Ala	His	Met	Asp	His	Asn	Phe	Met	Thr	Ala	Ser	Val	
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Pro	Thr	Phe	Arg	Leu	Asn	Phe	His	Val	Arg	Arg	Leu	Val	Asn	Ala	Gly	
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Val	Asp	Glu	Arg	Val	Lys	Ser	Glu	Thr	Leu	Gly	Cys	Gly	Ile	Glu	Met	
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Ser	Phe	Asp	Val	Arg	Val	Gly	Val	Val	Gly	Val	Glu	Ile	Ser	Thr	Gly	
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gaa	gtt	gtt	tat	gaa	gag	ttc	aat	gat	aat	ttc	atg	aga	agt	gga	tta	963
Glu	Val	Val	Tyr	Glu	Glu	Phe	Asn	Asp	Asn	Phe	Met	Arg	Ser	Gly	Leu	
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Lys Ala Thr Glu Phe Ile Ala Val Met Glu Ala Ile Leu Leu Ala Gly	
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Thr His Leu Ile Glu Leu Pro Val Asp Ser Lys Val Pro His Asn Trp	
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Val Lys Val Asn Ser Thr Lys Lys Thr Ile Arg Tyr His Pro Pro Glu	
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725 730 735	
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740 745 750	
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Cys Leu His Ser Leu Ser Thr Leu Ser Arg Asn Lys Asn Tyr Val Arg	
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Ser Val Gly Thr Tyr His Val Ser Tyr Leu Thr Leu Gln Lys Asp Lys	
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Pro Pro Pro Lys Ile Ser Ala Thr Val Ser Phe Ser Pro Ser Lys Arg

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Lys Leu Leu Ser Asp His Leu Ala Ala Ala Ser Pro Lys Lys Pro Lys

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Leu Ser Pro His Thr Gln Asn Pro Val Pro Asp Pro Asn Leu His Gln

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Arg Phe Leu Gln Arg Phe Leu Glu Pro Ser Pro Glu Glu Tyr Val Pro

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Glu Thr Ser Ser Ser Arg Lys Tyr Thr Pro Leu Glu Gln Gln Val Val

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Glu Leu Lys Ser Lys Tyr Pro Asp Val Val Leu Met Val Glu Val Gly

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Tyr Arg Tyr Arg Phe Phe Gly Glu Asp Ala Glu Ile Ala Ala Arg Val

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Leu Gly Ile Tyr Ala His Met Asp His Asn Phe Met Thr Ala Ser Val

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155

160

Pro Thr Phe Arg Leu Asn Phe His Val Arg Arg Leu Val Asn Ala Gly

165

170

175

Tyr Lys Ile Gly Val Val Lys Gln Thr Glu Thr Ala Ala Ile Lys Ser

180

185

190

His Gly Ala Asn Arg Thr Gly Pro Phe Phe Arg Gly Leu Ser Ala Leu

195

200

205

Tyr Thr Lys Ala Thr Leu Glu Ala Ala Glu Asp Ile Ser Gly Gly Cys

210

215

220

Gly Gly Glu Glu Gly Phe Gly Ser Gln Ser Asn Phe Leu Val Cys Val

225

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240

Val Asp Glu Arg Val Lys Ser Glu Thr Leu Gly Cys Gly Ile Glu Met

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255

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Pro Leu Ser Gln Gln Thr Glu Lys Phe Leu Val Ala Met Ala Gly Pro
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Thr Ser Asn Val Arg Val Glu Arg Ala Ser Leu Asp Cys Phe Ser Asn
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Val Lys Asn Asn Ser Asp Gly Ser Glu Ser Gly Ser Leu Phe His Asn
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17

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171

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Glu Gly Asp Ala Lys Gly Asp Ala Ser Val Arg Phe Ala Val Ser Lys	
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 225 230 235 240
 Asp Ala Asn Arg Arg Arg Pro Asp Asp Pro Leu Tyr Asp Arg Lys Thr
 245 250 255
 Leu His Ile Pro Pro Asp Val Phe Lys Lys Met Ser Ala Ser Gln Lys
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32

Arg Ser Asp Tyr Gln Pro Arg Gly Cys Asp Gln Asp Leu Val Phe Leu
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Glu Leu Arg Ser Glu Phe Ser Ser Leu His Glu Asp Trp Leu Lys Ser
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Leu Val Gly Ile Ser Arg Val Ala His Asn Asn Ala Pro Ile Gly Glu
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<400> 34

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<220>
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<400> 35

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<210> 36
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38

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<223> Reverse primer for PCR amplification of ATEAT1 SSLP marker in Arabidopsis thaliana subspecies

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<210> 44

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<223> Forward primer for PCR amplification of NGA63 SSLP marker in Arabidopsis thaliana subspecies

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<223> Reverse primer for PCR amplification of NGA63 SSLP marker in Arabidopsis thaliana subspecies

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in Arabidopsis thaliana subspecies

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<400> 82

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19

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<223> Forward primer for PCR amplification of NGA151 SSLP marker in
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<223> Forward primer for PCR amplification of NGA106 SSLP marker in
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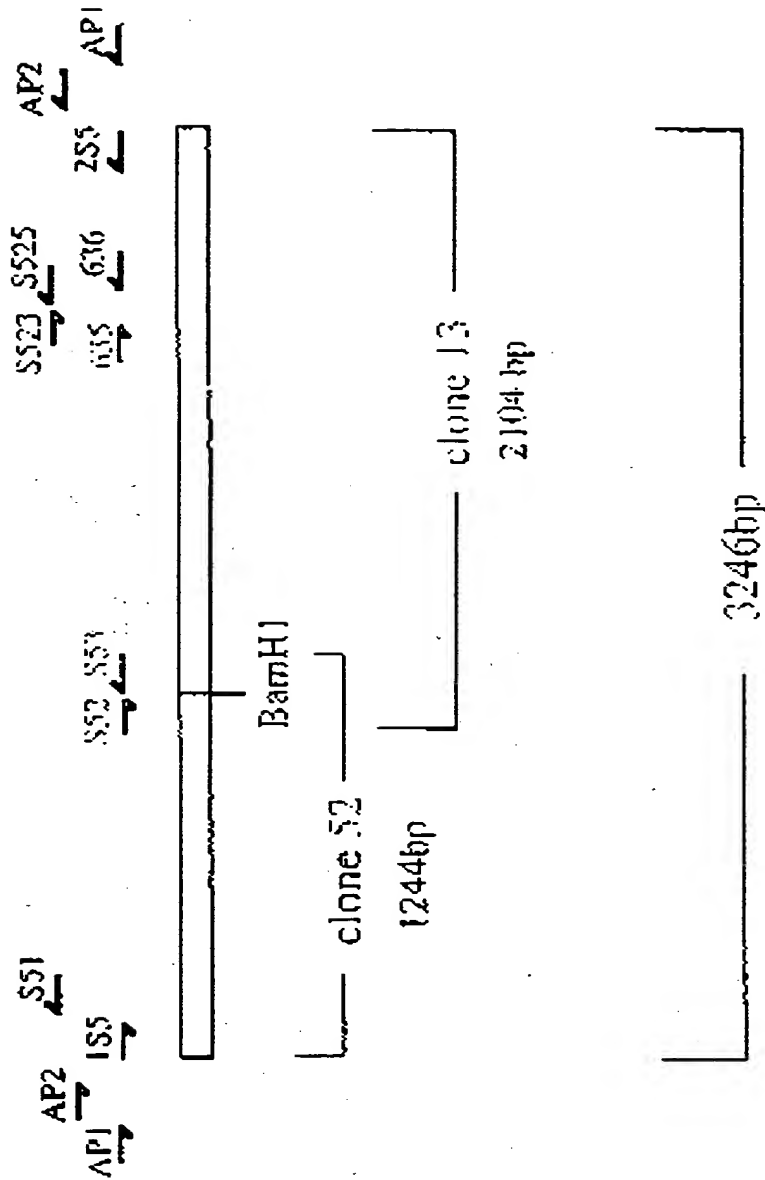
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accttagtgt tcataaacac aaactcacct gaatcaacac cacaagctaa acctaaatcc	7800
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Figure 1



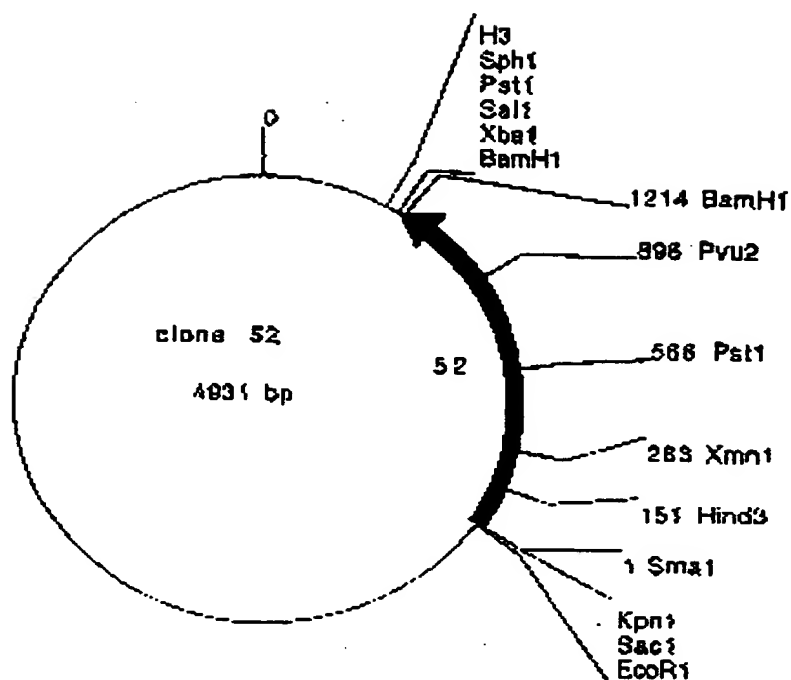


Figure 2

Comments/References: 52= 3' side of S5 (AIMSH3) 1244bp in pUC18/SmaI

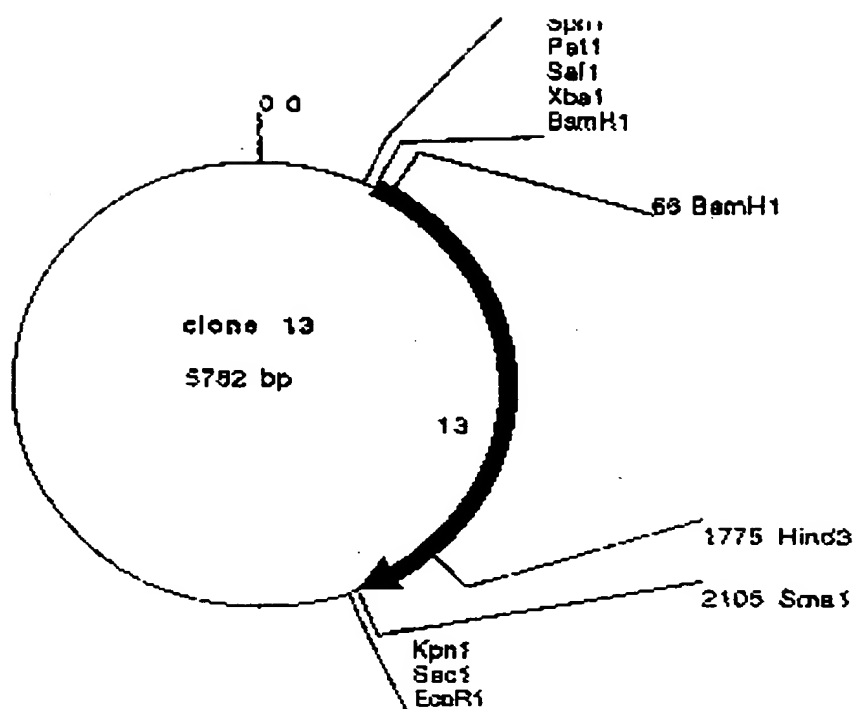


Figure 3

Comments/References: 13 = 3' side of S5 (AtMSH3) 2104bp in pUC18/SmaI

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925	TAT GAA GAG TTC AAT GAT AAT TTC ATG AGA AGT GGA TTA GAG GCT GTG AAT TTG AGC TTG	984
276	Y E E F N D N F M R S G L E A V I L S L	295
985	TCA CCA GCT GAG CTG TTG CTT GCC CAG CCT CTT TCA CAA CAA ACT GAG AAG TTT TTG GTG	1044
296	S P A E L L L G Q P L S Q Q T E K F L V	315
1045	GCA CAT GCT GGA CCT ACC TCA AAG GTT CGA GTG GGA CGT GCT TCA CTA GAT TGT TTC AGC	1104
316	A M A G P T E N V R V E R A S L D C F S	335
1105	AAT GGT AAT GCA GTA GAT GAG GTT AAT TCA TTA TGT GAA ABA ATC AGC GCA GGT AAT TTA	1164
336	N G N A V D E V I S L C E K I S A G N L	355
1165	GAA GAT GAT AAA GAA ATG AAG CTG GRG GCT GCT GAA AAG AAG AAT GCT TGC TTG ACA GTT	1224
356	E D D K E M K L E A A E K G N S C L T V	375
1225	CAT ACA AAT ATG AAC ATG CCA CAT CTG ACT GTT CAA GCC CTC GGC CTA AGC TTT TGC CMT	1284
376	H T I M N M P H L T V Q A L A L T F C M	395
1285	CTC AAA CAG TTT GGA TTT GAA AAG ATC GTT TAC CAA GGS GCT TCA TTT CGC TCT TTG TCA	1344
396	L K Q F G F E R I L Y Q G A S F R S L S	415
1345	AGT AAC ACA GAG ATG ACT CTC TCA GCC RAT ACT CTG CAA CAG TTG GAG GTT GTG AAA AAT	1404
416	S N T E M T L S R A N T L Q Q L E V V K N	435
1405	AAT TCA GAT GGA TCG GAA TCT GGC TCC TTA TTC CAT AAT ATG AAT CAC ACA CTT ACA GTA	1464
436	N S D G S E S G S L E H N K N H T L T V	455
1465	TAT GCT TCC AGG CTT CTT AGA CAC TCG GTG ACT CAT CTT CTA TCC GAT AGA AAT TTG ATA	1524
456	Y C S R L L L R H K V T H P L C D R N L I	475
1525	TCT GCT CGG CTT GAT GCT GTT TCT CAG AAT TCT GCT TCC ATG GGA TCT CAT AGT TCT TCC	1584
476	S A R L D A V S E I S A C N G S H S S S	495
1585	CAG CTC AGC AGT GAG TTG CTT GAA GGA CGT TCT GAG AGA GCA AAT GTA TCA CTT GAG TTT	1644
496	Q L S S E L V I E G S E R A I V S P E F	515
1645	TAT CTC GTG CTC TCC TCA GTC TTG ACA GCT ATG TCT AGA TCA TCT TCT GAT ATT CAA CGT GGA	1704
516	Y L V L S S V L T A K S R S S D I Q R G	535
1705	ATA ACA AGA ATC TTT CAT CGG ACT GCT AAA GCC ACA GAG TTC ATT GCA GTT ATG GAA GCT	1764
536	I T R I F H R T A K A T E F I A V M E A	555
1765	ATT TTA CTT GCG GAG AAG CAA AAT CAG CGG CTT GGC ATA AAG CAA GAC TCT GAA ATG AGS	1824
556	I L L A G K Q I Q R L G I K Q D S E M R	575

Figure 4 (Continued)

1825 576	AGT ATG CAA TCT GCA ACT GTG CGA TCT ACT CTT TTG AGA AAA TTG ATT TCT GTT ATT TCA	1884 595
1885 596	TCC CTT GTT GTG GAT GAC AAT GGC GGA AAT CTT CTC TCT GGC CTA AAT AAG GAA GCG GCT	1944 615
1945 616	GTT CGA GGT GAC TTG CTC GAC ATA CTA ATC ACT TCC AGC GAC CAA TTT CCT GAG CTT GCT	2004 635
2005 636	GAA GCT CGC CAA GCA GTT TTA GTC ATC AGG GAA AAG CTG GAT TCC TCG ATA GCT TCA TTT	2064 655
2065 656	CGC AAG AAG CTC GCT ATT CGA AAT TTG GAA TTT CTT CAA TTG TCG GGG ATC ACA CAT TTG	2124 675
2125 676	ATA GAG CTG CCC GTT GAT TCC AAG GTC CTT ATG AAT TCG GTG AAA GTA AAT AAG ACC AAG	2184 695
2185 696	AAG ACT AAT CGA TAT CAT CCC CCA GAA ATA ATA GAT TCG GAT TCG CTA GCT CTA GCA	2244 715
2245 716	ACT GAA CMT CTT GCC ATT GTG AAC CGA GCT TCG TGG GAT AGT TTC CTC AAG AGT TTC AGT	2304 735
2305 736	ACA TAC TAC ACA GAT TTT AAG GCT GCG GTT CAA GCT CTT GCT GCA CTG GAC TGT TTG CAC	2364 755
2365 756	TCC CTT TCA ACT CTA TCT AAT AAC AAG AAC TAT GTC CGT CCC GAG TTT GTG GAT GAC TGT	2424 775
2425 776	GAA CCA GTT GAG ATA AAC ATA CAG TCT GGT CGT CAT CCT GTA CTG GAG ACT ATA TTA CAA	2484 795
2485 796	GAT AAC TTC GTC CCA AAT GAC ACA ATT TTG CAT GCA GAA GGG GAA TAT TGC CAA ATT ATC	2544 815
2545 816	ACC GGA CCT AAC ATG GGA GGA AAG ACC TGC TAT ATC CGT CAA GTT GCT TTA ATT TCC ATA	2604 835
2605 836	ATG CCT CAG GTT GGT TCC TTT GTA CCA GCG TCA TTC GCG AAG CTG CAC GTG CTT GAT GGT	2664 855
2665 856	GTT TTC ACT CCG ATG GGT GCT TCA GAC AGT ATC CAG CAT GGC AGA AGT ACC TTT CTA GAA	2724 875

Figure 4 (Continued)

2725 GAA TTA AGT GAA GCG TCA CAC ATA ATC AGA ACC TGT TCT TCT CGT TCG CTT GTT ATA TTA 2784
 876 E L E E A S H I I R T C S S R S L V I L 895
 2785 GAT GAG CTT GGA AGA GGC ACT AGC ACA CAC GGT GTA GCC AAT GCC TAT GCA ACA TTA 2846
 896 D E L G R G T S T H D G V R I A Y A T L 915
 2845 CAG CAT CTC CTA GCA GAA AAG AGA TGT TTG GTT CTT TTT GTC ACG CAT TAC CCF GAA ATA 2904
 916 Q H L L A E K R C L V L F V T H Y P E I 935
 2905 GCT GAG ATC AGT AAC CGA TTC CCA GGT TCT GTT GGS ACA TAC CAT CTC TCG TAT CTG ACA 2964
 936 A E I S N G F P G S V G T Y H V S Y L T 955
 2965 TTG CAG AAG GAT AAA GGC AGT TAT GAT CAT GAT GAT GTG ACC TAC CTA TAT AAG CTT GTG 3024
 956 L Q K D K G S Y D H D V T Y L Y K L V 975
 3025 CGT GGT CTT TCG ACC AGG AGC TTT GGT TTT AAG GTT GGT CAG CTT GCG CAG ATA COT CCA 3084
 976 R G L C S R S F C F K V A Q I P P 995
 3085 TCA TGT ATA CGT CGA GCC ATT TCA ATG CCT GCA AAT TTG GAA GCT GAG GTA CGT GCA AGA 3144
 996 S C I R A I S K A A K L E A E V R A R 1015
 3145 GAG AGA AAT ACA CGC ATG GGA GAA CCA GAA GCA CAT GAA GAA CCG AGA GCG GCA GAA GAA 3204
 1016 E R N T R K G E P E G H E E P R G A E E 1035
 3205 TCT ATT TCG GCT CTA GGT GAC TTG TTT GCA GAC CTG AAA TTT GCT CTC TCT GAA GAG GAC 3264
 1036 S I S A L G D L F A D L K F A L S E E D 1055
 3265 CCT TGG AAA GCA TTC GAG TTT TTA AAG CBT GCT TCG AAG AAT GCT GGC AAA ATC AGA CTA 3324
 1056 P W K A F E F L K H A N K I A G K I R L 1075
 3325 AAA CCA ACT TGT TCA TTT TGA TTATATCTTACATATATAGCAAGCTCTTGATCATCTGTAGTTGG 3397
 1076 K F T C S F * 1082
 3398 TACTACCTT ATG TGT ATT AGT ATA ACA AGA AAA GAG AAT TNG AGAG ATG GAT TCT AAT CCG 3458
 1 H C I S I T R K E N N O S N P 5
 3459 GTG TTG CAG TAC ATC TTT TCT CCA CCC GCA TAA AAAAATAAATAAATAAATAAATAAATAA 3522
 6 V L Q Y I F S P P A * 16

Figure 4 (Continued)

Figure 5

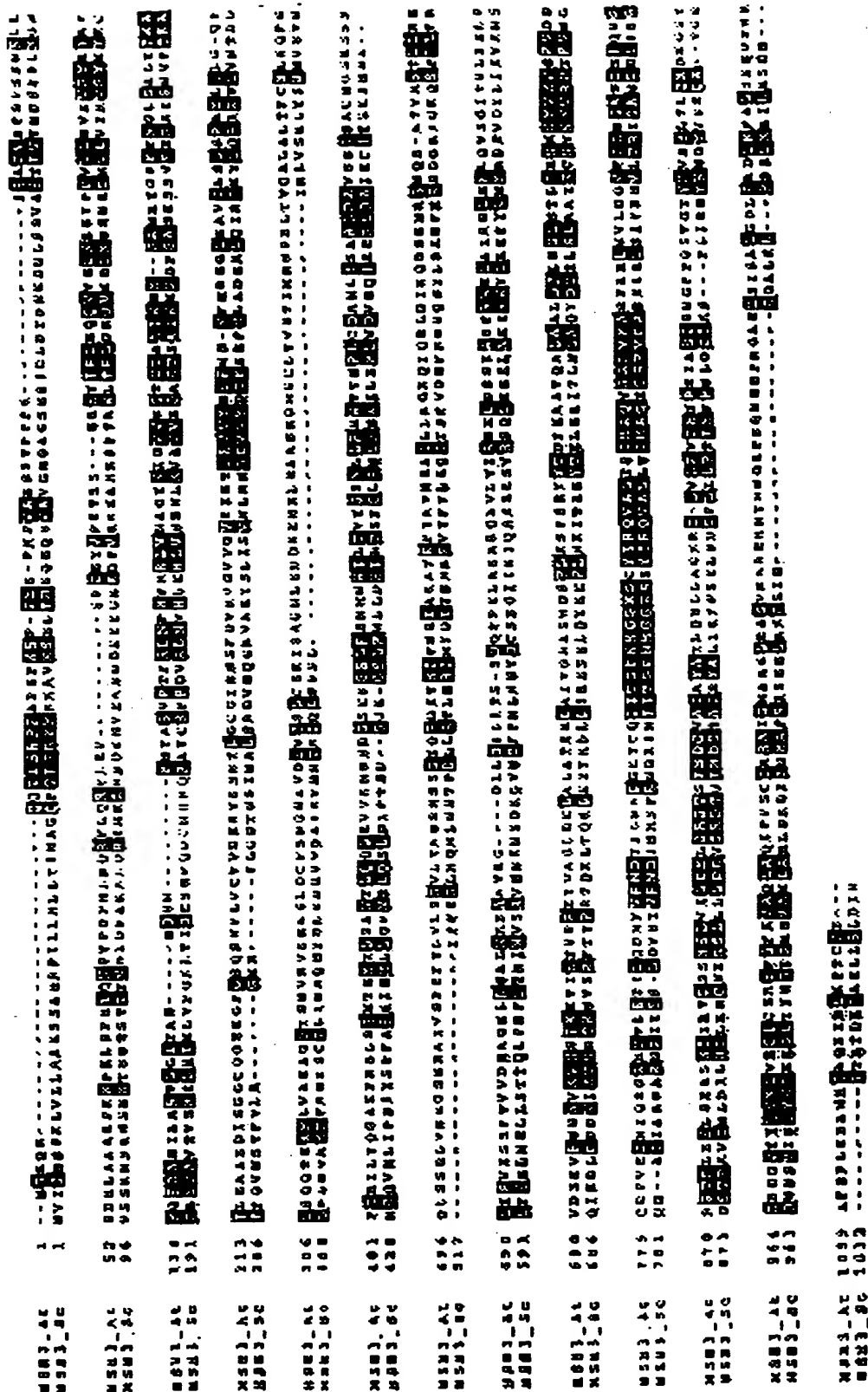
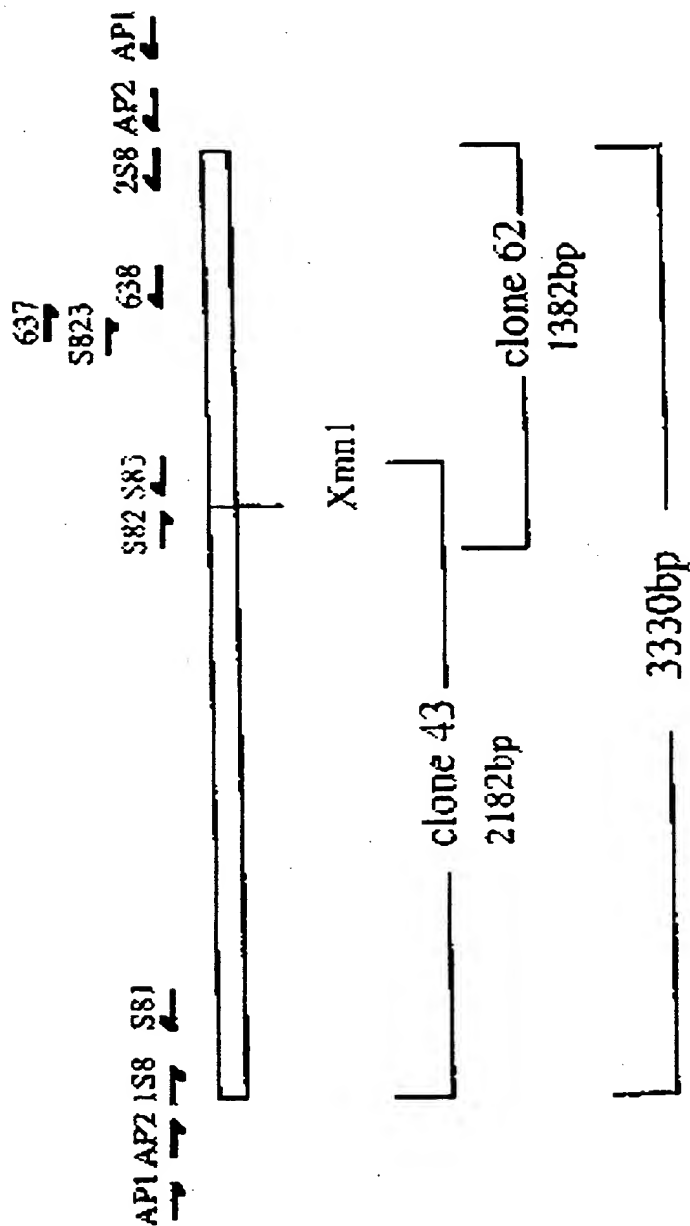
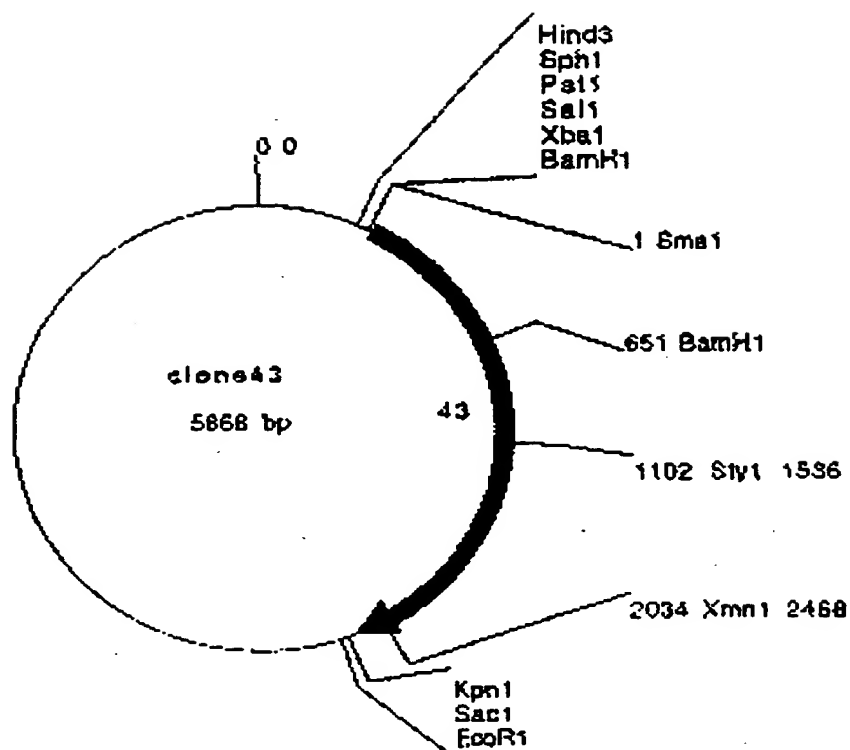
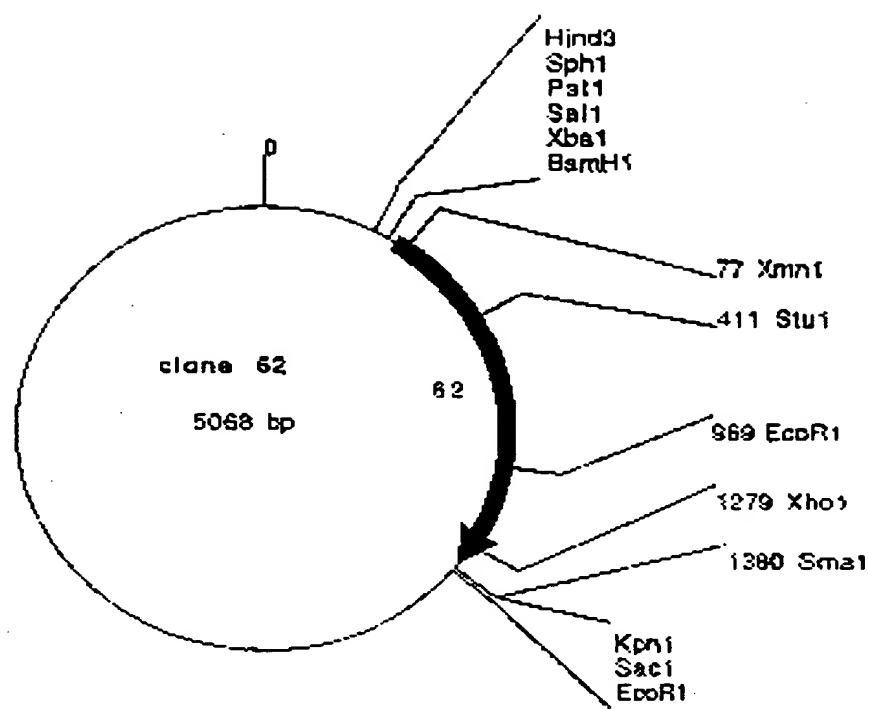


Figure 6



**Figure 7**

Comments/References: 43= 5' side of S8 (AIMSH6) 2182 bp in pUC18/Sma1

**Figure 8**

Comments/References: 62= 3' side of S8 (AIMSH6) 1378bp in pUC18/Sma1

Figure 9

Figure 9 (Continued)

18934	ATA	TTT	AAC	AAT	AGC	TGT	GAT	GGT	GGT	CCT	TCA	GGG	ACC	TTG	TAC	AAA	TAT	CTT	GAT	AAC	1893
565	I	F	N	N	S	C	D	G	G	P	S	G	T	L	Y	K	Y	L	D	N	584
1894	TGT	GTT	AGT	CCA	ACT	GGT	AAG	CGA	CTC	TTA	AGG	AAT	TGG	ATC	TGC	CAT	CCA	CTC	AAA	GAT	1953
585	C	V	S	P	T	G	K	R	L	L	R	N	W	I	C	H	P	L	K	D	604
1954	GTA	GAA	AGC	ATC	AAT	AAA	CGG	CTT	GAT	GTA	GTT	GAA	GAA	TTC	ACG	GCA	AAC	TCA	GAA	AGT	2013
605	V	E	S	I	N	K	R	L	D	V	V	E	L	F	I	A	N	S	E	S	624
2014	ATG	CAA	ATC	ACT	GGC	CAG	TAT	CTC	CAC	AAA	CTT	CCA	GAC	TTA	GAA	AGA	CTG	CTC	GGA	CGC	2073
625	K	Q	I	T	G	Q	Y	L	S	K	L	P	D	L	E	R	L	L	G	R	644
2074	ATC	AAG	TCT	AGC	GTT	CGA	TCA	TCA	CCC	TCT	GTC	TTC	GCT	GCT	GCT	CTG	GGG	AAA	AAA	GTG	2133
645	I	K	S	S	V	R	S	S	A	S	V	L	P	A	L	L	G	K	K	V	664
2134	CTG	AAA	CAA	CGA	GTT	AAA	UGA	TTT	GGG	CAA	ATC	CTG	AAA	ATC	TTC	AGA	AGT	GGA	ATT	GAT	2193
665	L	K	Q	R	V	K	A	F	G	Q	I	V	K	S	F	R	S	G	I	E	684
2194	CTG	TTG	TTG	GCT	CTA	CAG	AGG	GAA	TCA	AAT	ATG	ATG	ATG	ATG	ATG	TAT	AAT	CTC	TGT	AAA	2253
685	L	L	L	A	L	Q	K	E	S	N	M	R	S	L	L	Y	K	L	C	K	704
2254	CCT	CCT	ATA	TTA	GTA	GGA	AAA	AGC	GGG	CTA	GAG	TTA	TTT	CTT	TCT	CAA	TTC	GAA	GCA	GCC	2313
705	L	P	I	L	V	G	K	S	G	L	E	L	F	L	S	Q	E	E	A	A	724
2314	ATA	GAT	AGC	GAC	TTT	CCA	AAT	TAT	CAG	AAC	CAE	GAT	GTG	ACA	GAT	GAA	AAC	GCT	GAA	ACT	2373
725	I	D	S	D	F	P	N	Y	Q	N	Q	D	V	T	D	E	N	A	E	T	744
2374	CTC	ACA	ATA	CTT	ATC	GAA	CTT	TTT	ATC	GAA	ACA	GCA	ACT	CAA	TGG	TCT	GAG	GTG	ATT	CAC	2493
745	L	T	I	L	I	E	L	E	I	E	R	A	T	Q	W	S	E	V	I	H	764
2434	ACC	ATA	AGC	TGC	CTA	GAT	GTC	CTG	AGA	TCT	TTT	GCA	ATC	GCA	GCA	AGT	CTC	TCT	GCT	GGA	2493
765	T	I	S	C	L	D	V	L	P	S	F	A	I	A	A	S	L	S	A	G	784
2494	AGC	ATG	CCC	AGG	CCT	GTT	ATT	TTT	CCC	GAA	TCA	GAA	GCT	ACA	CAT	CAG	AAT	CAG	AAA	ACA	2553
785	S	M	A	R	P	V	I	F	P	E	S	E	A	T	D	Q	N	Q	K	T	804
2554	AAA	GEG	CCA	ATA	CTT	AAA	ATC	CAA	GGA	CTA	TGG	CAT	CCA	TTT	GCA	GTT	GCA	GCC	GAT	GGT	2613
805	K	G	P	I	L	K	I	Q	G	L	W	H	P	F	A	V	A	A	D	G	824
2614	CAA	TTG	CCT	GTT	CCG	AAT	GAT	ATA	CTC	CTT	GGC	GAG	GCT	AGA	AGA	AGC	AGT	GGC	AGC	ATT	2673
825	Q	L	P	V	P	N	O	I	L	L	G	E	A	R	R	S	S	G	S	I	844
2674	CAT	CCT	CGG	TCA	TTG	TTA	CTG	ACG	GGA	CCA	AAA	ATG	GGC	GGA	AAA	TCA	ACT	CTT	CTT	CGT	2733
845	H	P	R	S	L	L	L	L	T	G	P	N	M	G	K	S	T	L	L	R	864

Figure 9 (Continued)

2734 865	GCA ACA TGT CTG GGC GTT ATC TTT GGC CBA CTT GGC TGC TAC GTG CCG TGT GAG TCT TGC	2793 884
2794 885	GAA ATC TCC CTC GTG GAT ACT ATC TTC ACA AGG CTT GGC GCA ICT GAT AGA ATC ATG ACA	2853 904
2854 905	GGA GAG AGT ACC TTT TTG GTA GAA TGC ACT GAG ACA GCG TCA GTT CTT CAG AAT GCA ACT	2913 924
2914 925	CAG GAT TCA CTA GTA ATC CTT GAC GAA CTG GGC AGA GGA ACT AGT ACT TTC GAT GGA TAC	2973 944
2974 945	GCC ATT GCA TAC TCG GTT TTT CGT CAC CTG GTA GAG AAA GTT CAA TGT CGG ATG CTC TTT	3033 964
3034 965	GCA ACA CAT TAC CAC CCT CTC ACC AAG GAA TTG GCG TCT GAC CCA CGT GTC ACC TCG AAA	3093 984
3094 985	CAC ATG CCT TGC GCA TTC ABA TCA AGA TCT GAT TAT CAA CCA CCA CCA CCA CCA CCA CCA	3153 1004
3154 1005	CTA GTG TTC TTG TAC CGT TTA ACC GAG GGA GGT TGT CCA CCA CCA CCA CCA CCA CCA	3213 1024
3214 1025	GCA CTC ATG GCT GGA ATA CCA AAC CAA GTS GTT GAA ACA GCA TCA GGT GGT CCA GGC	3273 1044
3274 1045	ATG AAG AGA TCA AAT GGG GGA AAC TTC AAG TCA AGT GAG CTA AGA TCT GAG TTC TCA AGT	3333 1064
3334 1065	CTG CAT GAA GAC TGG CTC AAG TCA TTG GTG CGT ATT TCT CGA GTC GCC CAC AAG AAT GGC	3393 1084
3394 1085	CCC ATT GGC GAA GAT GAC TAC GAC ACT TTG TTT TGC TTA TGG CAT GAG ATC AAA TCC TCT	3453 1104
3454 1105	TAC TGT GTT CCC AAA TAA ATG GGT ATG ACA TAA CACTATCTGAGCTGTTAAGTCTTTGCTCTCT	3521 5
3522 1	G RTG TTT ATT OCT CTT AAA AAA TGC TTA TAT ATC AAA AAA TGG TTT CCT CGA TTA AAA	3579 19
3580 20	AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA	3606 28

Figure 9 (Continued)

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CTATATATAAAGAAATGAAAGATATATATTGTTTTTTCATTTATCAAC	100
APACACCAAEACTTTTTTTTACCTTTTACATTEGTCACCAATACPA	150
GATAACGACATCGTTTAAATCATTTCCTAATTTTACCCCTAAGTTTACA	200
CCTAGAACCTTCTCCATCTTCGCAAGCACAGCCTGATTAGGACAGCTTT	250
ACCATTCTCATATTCTGAACTACCTGAGTCCTCTCATTEATCTGTTTCG	300
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Figure 11

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Figure 11 (Continued)

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Figure 11 (Continued)

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Figure 11 (Continued)

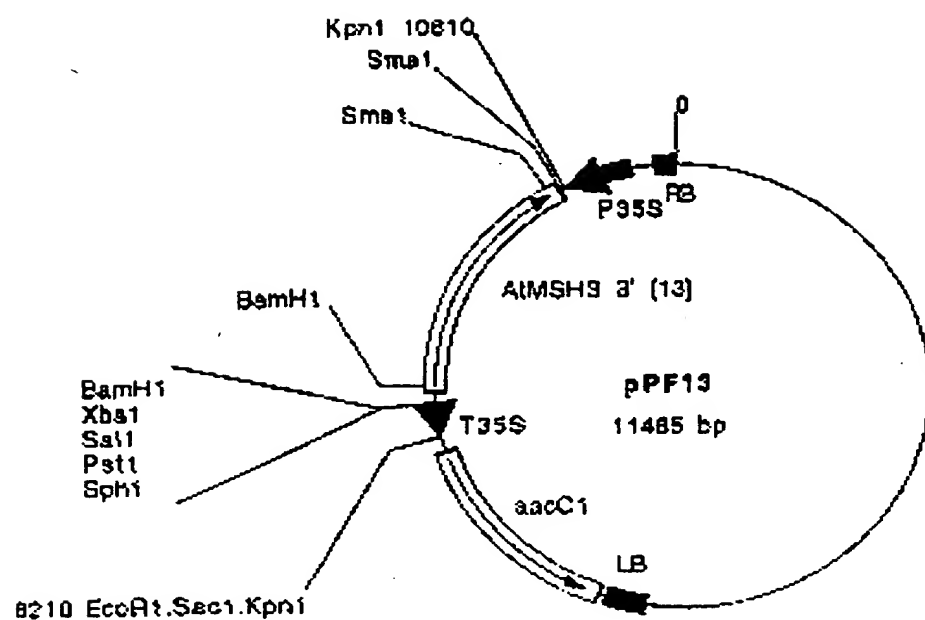


Figure 12

Comments/References: AIMSH3 3' side antisense : AIMSH3 3' (13 = 2104bp) from pUC18/13 SalI/SalI/T4 Into pCW184 BamHI/T4 In *Agrobacterium* LBA4404

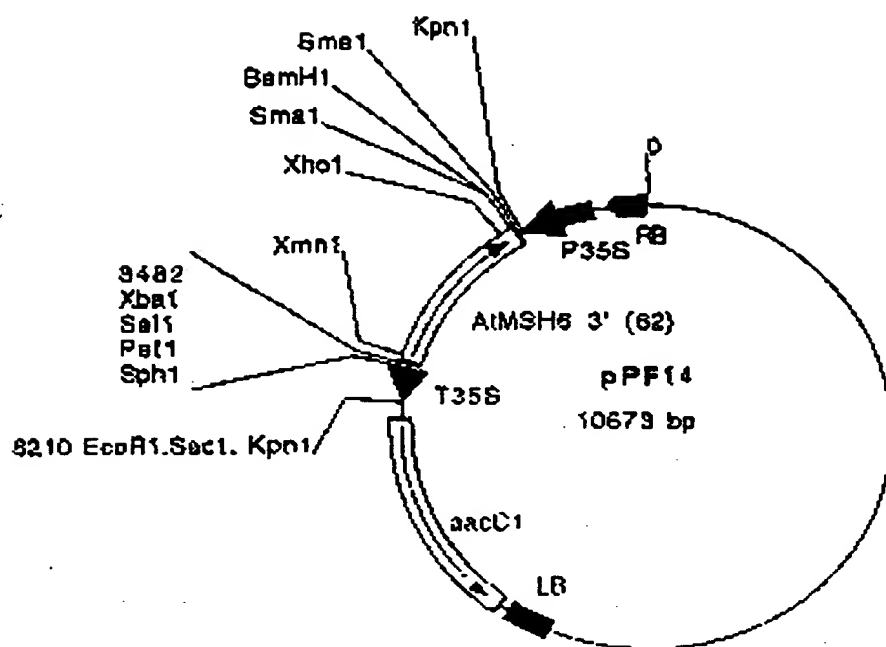


Figure 13

Comments/References: AtMSH6 (S8) 3' side antisens : 62 SalI/SalI/T4 (1379bp)
into pCW164 BamHI/T4

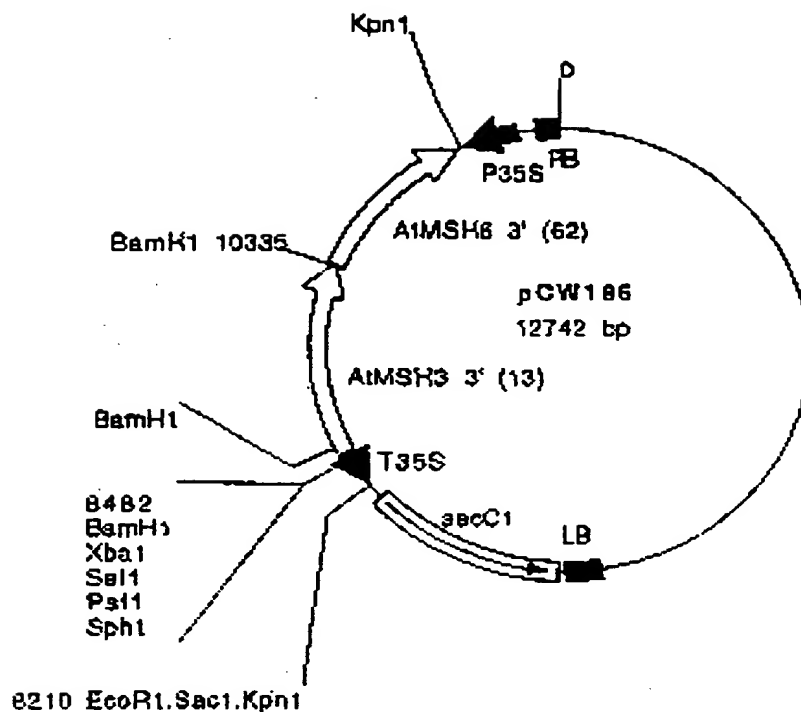


Figure 14

Comments/References: A1MSH6 3'/A1MSH3 3' antisense : A1MSH6 (SB) 3' side (62=1379bp)
Sal1/Sal1/T4 Into pPF13 (pCW184 A1MSH3 (S5) 3' side (13=2104) antisense)/SmaI. in
LBA4404

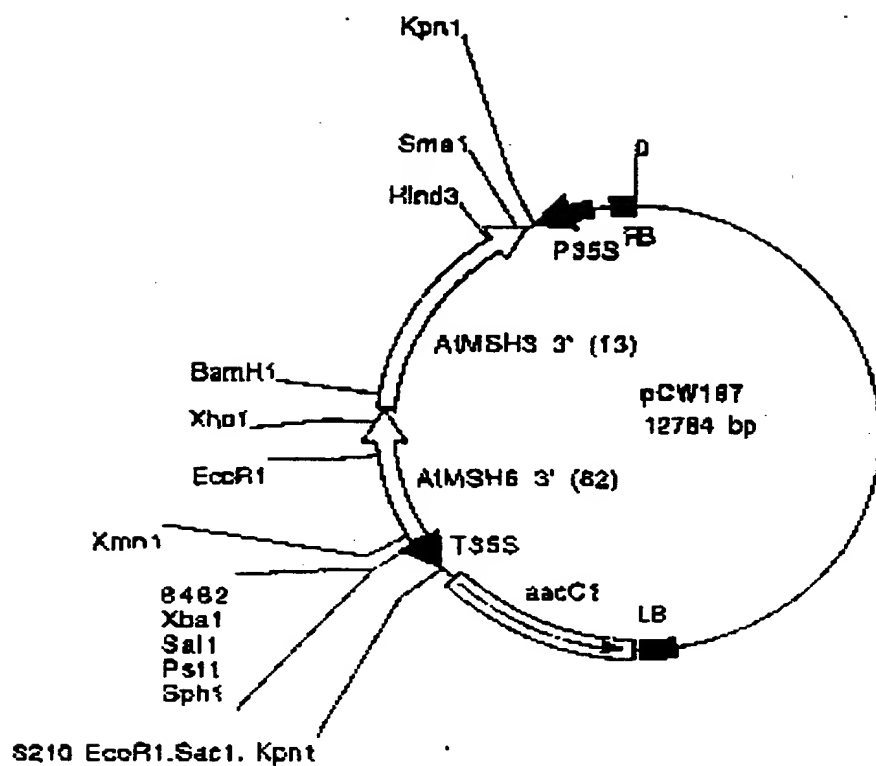


Figure 15

Comments/References: AtMSH3 3'/AtMSH6 3' antisense (D) : AtMSH3 (35) 3' side (13=2104bp) SalI/SstI/T4 into pPF14 (AtMSH6 (35) 3' side (62=1379bp) antisense into pCW184/SmaI. In LBA4404

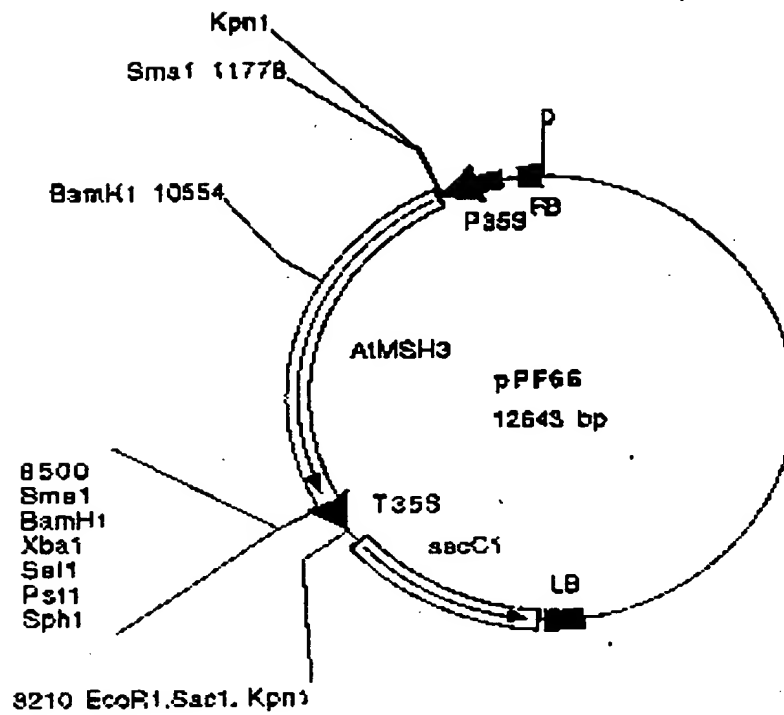


Figure 16

Comments/References: AIMSH3 (SB) complete, sense orientation : pPF25 (3342bp)
 SmaI into pCW164 SmaI

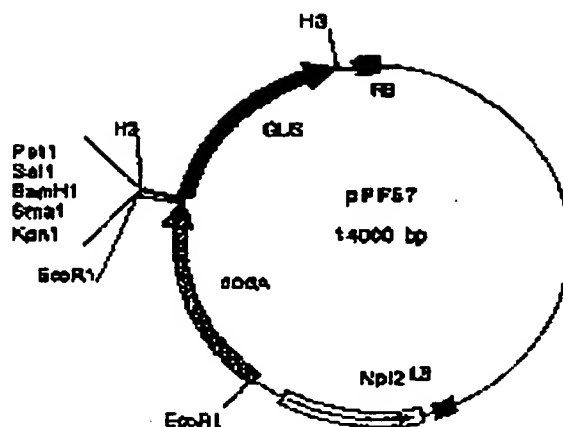


Figure 17

Comments/References: pPZP151 with codA EcoRI cassette in EcoRI site and HindIII GUS cassette in HindIII site, KanR. All genes under Promoter/terminator 35S

Figure 18

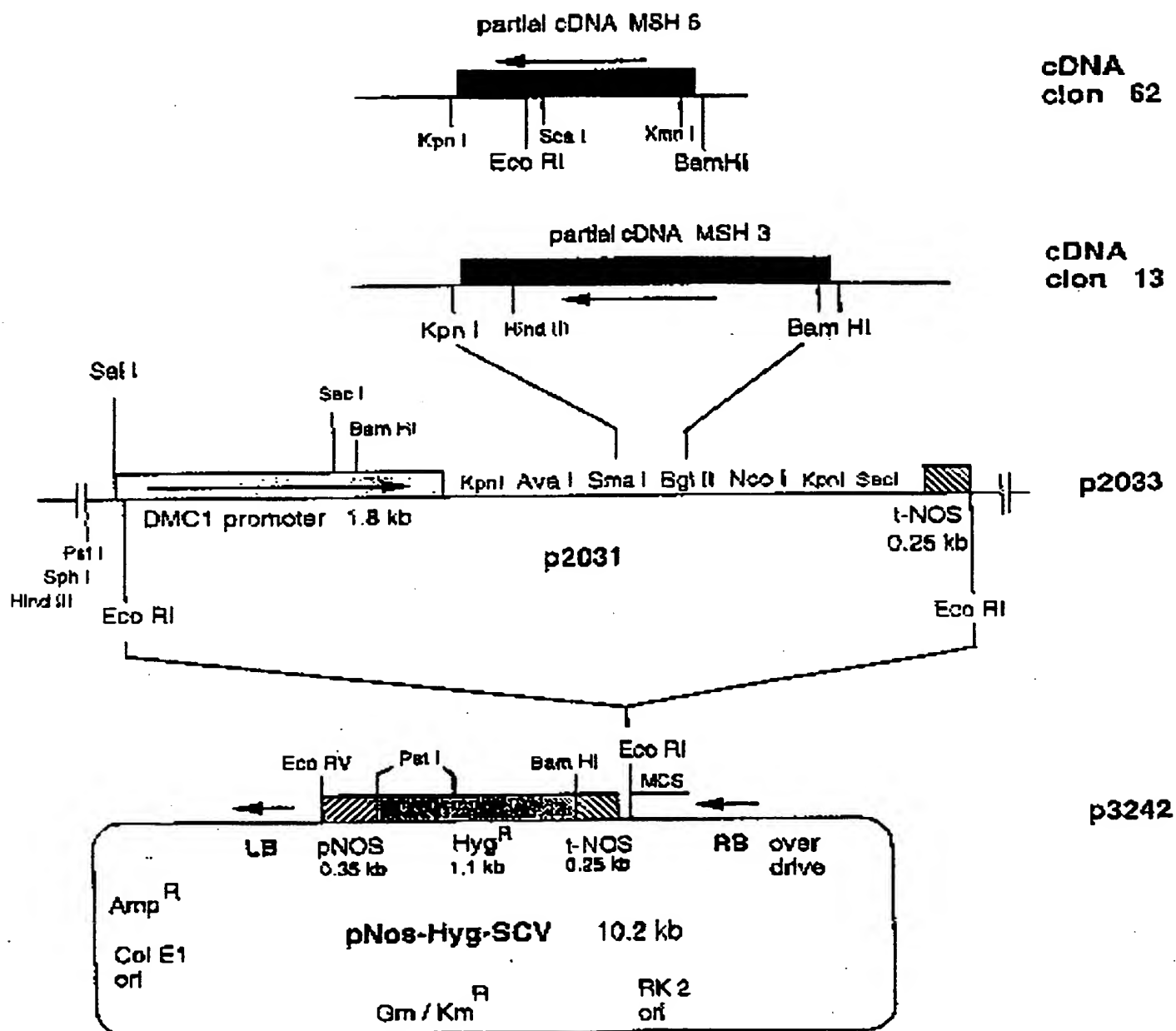
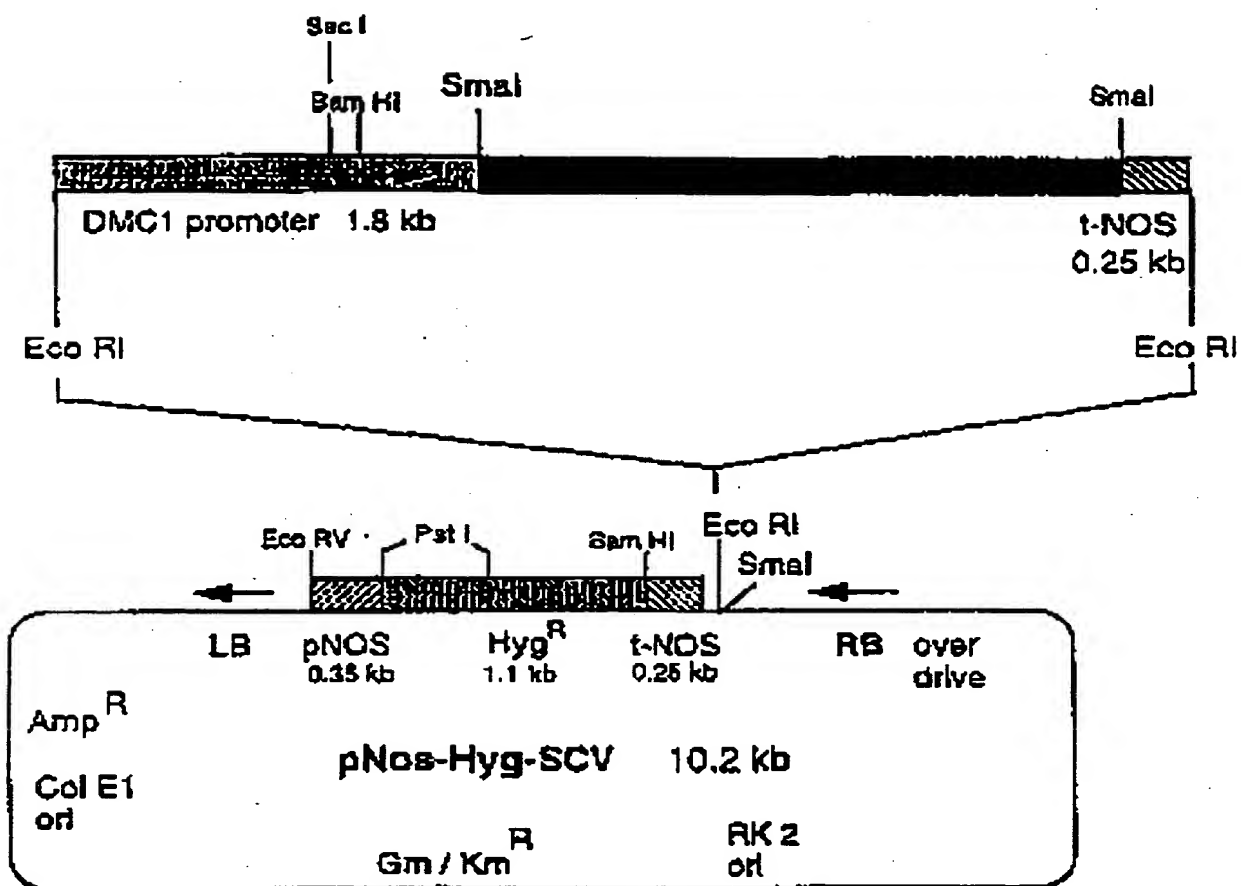


Figure 19**p3243**

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9

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Ser	Phe	Asp	Val	Arg	Val	Gly	Val	Val	Gly	Val	Glu	Ile	Ser	Thr	Gly	
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Glu	Val	Val	Tyr	Glu	Glu	Phe	Asn	Asp	Asn	Phe	Met	Arg	Ser	Gly	Leu	
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10

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Thr	His	Leu	Ile	Glu	Leu	Pro	Val	Asp	Ser	Lys	Val	Pro	His	Asn	Trp	675	680	685	
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Ile	Val	Asn	Arg	Ala	Ser	Trp	Asp	Ser	Phe	Leu	Lys	Ser	Phe	Ser	Arg	725	730	735	
Tyr	Tyr	Thr	Asp	Phe	Lys	Ala	Ala	Val	Gln	Ala	Leu	Ala	Ala	Leu	Asp	740	745	750	
Cys	Leu	His	Ser	Leu	Ser	Thr	Leu	Ser	Arg	Asn	Lys	Asn	Tyr	Val	Arg	755	760	765	
Pro	Glu	Phe	Val	Asp	Asp	Cys	Glu	Pro	Val	Glu	Ile	Asn	Ile	Gln	Ser	770	775	780	
Gly	Arg	His	Pro	Val	Leu	Glu	Thr	Ile	Leu	Gln	Asp	Asn	Phe	Val	Pro	785	790	795	800
Asn	Asp	Thr	Ile	Leu	His	Ala	Glu	Gly	Glu	Tyr	Cys	Gln	Ile	Ile	Thr	805	810	815	
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27

<210> 26

<211> 1385

<212> DNA

<213> Arabidopsis thaliana ecotype Columbia

<223> Clone 43

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Phe Gln Lys Pro Thr Ala Ala Thr Thr Lys Gly Leu Val Ser Gly Asp	
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Ala Ala Ser Gly Gly Gly Gly Ser Gly Gly Pro Arg Phe Asn Val Arg	
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Glu Gly Asp Ala Lys Gly Asp Ala Ser Val Arg Phe Ala Val Ser Lys	
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Ser Val Asp Glu Val Arg Gly Thr Asp Thr Pro Pro Glu Lys Val Pro	
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Arg Arg Val Leu Pro Ser Gly Phe Lys Pro Ala Glu Ser Ala Gly Asp	
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2715

2763

3811

2859

2907

2955

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305:

309

314'

319.

324

329

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Leu Leu Ala Arg Gly Tyr Lys Val Gly Arg Ile Glu Gln Leu Glu Thr
340 345 350

Ser Asp Gln Ala Lys Ala Arg Gly Ala Asn Thr Ile Ile Pro Arg Lys
355 360 365

Leu Val Gln Val Leu Thr Pro Ser Thr Ala Ser Glu Gly Asn Ile Gly
370 375 380

Pro Asp Ala Val His Leu Leu Ala Ile Lys Glu Ile Lys Met Glu Leu
385 390 395 400

Gln Lys Cys Ser Thr Val Tyr Gly Phe Ala Phe Val Asp Cys Ala Ala
405 410 415

Leu Arg Phe Trp Val Gly Ser Ile Ser Asp Asp Ala Ser Cys Ala Ala
 420 425 430
 Leu Gly Ala Leu Leu Met Gln Val Ser Pro Lys Glu Val Leu Tyr Asp
 435 440 445
 Ser Lys Gly Leu Ser Arg Glu Ala Gln Lys Ala Leu Arg Lys Tyr Thr
 450 455 460
 Leu Thr Gly Ser Thr Ala Val Gln Leu Ala Pro Val Pro Gln Val Met
 465 470 475 480
 Gly Asp Thr Asp Ala Ala Gly Val Arg Asn Ile Ile Glu Ser Asn Gly
 485 490 495
 Tyr Phe Lys Gly Ser Ser Glu Ser Trp Asn Cys Ala Val Asp Gly Leu
 500 505 510
 Asn Glu Cys Asp Val Ala Leu Ser Ala Leu Gly Glu Leu Ile Asn His
 515 520 525
 Leu Ser Arg Leu Lys Leu Glu Asp Val Leu Lys His Gly Asp Ile Phe
 530 535 540
 Pro Tyr Gln Val Tyr Arg Gly Cys Leu Arg Ile Asp Gly Gln Thr Met
 545 550 555 560
 Val Asn Leu Glu Ile Phe Asn Asn Ser Cys Asp Gly Gly Pro Ser Gly
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 Thr Leu Tyr Lys Tyr Leu Asp Asn Cys Val Ser Pro Thr Gly Lys Arg
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 595 600 605
 Asn Lys Arg Leu Asp Val Val Glu Glu Phe Thr Ala Asn Ser Glu Ser
 610 615 620
 Met Gln Ile Thr Gly Gln Tyr Leu His Lys Leu Pro Asp Leu Glu Arg
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 645 650 655
 Pro Ala Leu Leu Gly Lys Lys Val Leu Lys Gln Arg Val Lys Ala Phe
 660 665 670
 Gly Gln Ile Val Lys Gly Phe Arg Ser Gly Il Asp Leu Leu Leu Ala
 675 680 685
 Leu Gln Lys Glu Ser Asn Met Met Ser Leu Leu Tyr Lys Leu Cys Lys
 690 695 700

Leu Pro Ile Leu Val Gly Lys Ser Gly Leu Glu Leu Phe Leu Ser Gln
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 Phe Glu Ala Ala Ile Asp Ser Asp Phe Pro Asn Tyr Gln Asn Gln Asp
 725 730 735
 Val Thr Asp Glu Asn Ala Glu Thr Leu Thr Ile Leu Ile Glu Leu Phe
 740 745 750
 Ile Glu Arg Ala Thr Gln Trp Ser Glu Val Ile His Thr Ile Ser Cys
 755 760 765
 Leu Asp Val Leu Arg Ser Phe Ala Ile Ala Ala Ser Leu Ser Ala Gly
 770 775 780
 Ser Met Ala Arg Pro Val Ile Phe Pro Glu Ser Glu Ala Thr Asp Gln
 785 790 795 800
 Asn Gln Lys Thr Lys Gly Pro Ile Leu Lys Ile Gln Gly Leu Trp His
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 Pro Phe Ala Val Ala Ala Asp Gly Gln Leu Pro Val Pro Asn Asp Ile
 820 825 830
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 835 840 845
 Leu Leu Leu Thr Gly Pro Asn Met Gly Gly Lys Ser Thr Leu Leu Arg
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 Ala Thr Cys Leu Ala Val Ile Phe Ala Gln Leu Gly Cys Tyr Val Pro
 865 870 875 880
 Cys Glu Ser Cys Glu Ile Ser Leu Val Asp Thr Ile Phe Thr Arg Leu
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 Gly Ala Ser Asp Arg Ile Met Thr Gly Glu Ser Thr Phe Leu Val Glu
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 Cys Thr Glu Thr Ala Ser Val Leu Gln Asn Ala Thr Gln Asp Ser Leu
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 Val Ile Leu Asp Glu Leu Gly Arg Gly Thr Ser Thr Phe Asp Gly Tyr
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 Ala Ile Ala Tyr Ser Val Phe Arg His Leu Val Glu Lys Val Gln Cys
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 Arg Met Leu Phe Ala Thr His Tyr His Pro Leu Thr Lys Glu Phe Ala
 965 970 975
 Ser His Pro Arg Val Thr Ser Lys His Met Ala Cys Ala Phe Lys Ser
 980 985 990

32

Arg Ser Asp Tyr Gln Pro Arg Gly Cys Asp Gln Asp Leu Val Phe Leu
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 1010 1015 1020
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 1060 1065 1070
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 microsatellite
 <400> 32

accatgcata gcttaaactt cttg

24

<210> 33
 <211> 22
 <212> DNA
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 <223> Reverse primer for PCR amplification of ATHGENEA
 microsatellite
 <400> 33

acataaccac aaatagggtt gc

22

33

<210> 34
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<220>
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gaagcgatat cgttcgtg

18

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<400> 35

agattcgag aacattcc

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acgcgtcgac tcagctatga gattactcgt g

31

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gctctagatc tctcgtctta agactctct

29

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<220>
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32

<210> 39
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<220>
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48

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<220>
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26

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<400> 41

cccatggaga tctccgggt accgatctgc ttcgaggg

38

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Arabidopsis thaliana subspecies

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Arabidopsis thaliana subspecies

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<210> 44
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Arabidopsis thaliana subspecies

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aaccaaggca cagaagcg

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<210> 45
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Arabidopsis thaliana subspecies

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acccaagtga tggccacc

18

<210> 46

<211> 21

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21

<210> 47

<211> 22

<212> DNA

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<223> Reverse primer for PCR amplification of NGA248 SSLP marker in *Arabidopsis thaliana* subspecies

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tctgtatctc ggtgaattct cc

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<210> 48

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<212> DNA

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ggtctgttga cgtcgtaagt cg

22

<210> 49

<211> 22

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<210> 50

<211> 22

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<223> Forward primer for PCR amplification of NGA280 SSLP marker in *Arabidopsis thaliana* subspecies

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ctgatctcac ggacaatagt gc 22

<210> 51

<211> 20

<212> DNA

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<223> Reverse primer for PCR amplification of NGA280 SSLP marker in *Arabidopsis thaliana* subspecies

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ctccagtgg aagctaaagg g 21

<210> 53

<211> 21

<212> DNA

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tgtttttttag gacaaatggc g 21

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ccttcacatc caaaacccac 20

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gcacataccc acaaccagaa 20

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in Arabidopsis thaliana subspecies

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cgtcacgctt ctccgtaaag 20

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in Arabidopsis thaliana subspecies

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Arabidopsis thaliana subspecies

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20

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Arabidopsis thaliana subspecies

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acstatcaat atattaaagt agc

23

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Arabidopsis thaliana subspecies

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18

<210> 61

<211> 22

<212> DNA

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gaggacatgt ataggagct cg

22

<210> 62

<211> 20

<212> DNA

<213> Artificial sequence

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<223> Forward primer for PCR amplification of AthBIO2 SSLP marker in *Arabidopsis thaliana* subspecies

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tgacctccc ttccatggag

20

<210> 63

<211> 22

<212> DNA

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ttaacagaaa cccaagctt tc

22

<210> 64

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<223> Forward primer for PCR amplification of AthUBIQUE SSLP marker in *Arabidopsis thaliana* subspecies

<400> 64

aggcaaatgt ccatttcatt g

21

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<223> Reverse primer for PCR amplification of ACHUBIQUE SSLP marker in Arabidopsis thaliana subspecies

<400> 65

acgacatggc agatttctcc

20

<210> 66

<211> 21

<212> DNA

<213> Artificial sequence

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<223> Forward primer for PCR amplification of NGA172 SSLP marker in Arabidopsis thaliana subspecies

<400> 66

agctgcttcc ttatagcgtc c

21

<210> 67

<211> 19

<212> DNA

<213> Artificial sequence

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<400> 67

catccgaatg ccattgttc

19

<210> 68

<211> 21

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<223> Forward primer for PCR amplification of NGA126 SSLP marker in
Arabidopsis thaliana subspecies

<400> 58

gaaaaaacgc tactttcgtg g

21

<210> 69

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caagagcaat atcaagagca gc

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<210> 70

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<223> Forward primer for PCR amplification of NGA162 SSLP marker in
Arabidopsis thaliana subspecies

<400> 70

catgcaattt gcatctgagg

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<210> 71

<211> 22

<212> DNA

<213> Artificial sequence

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<223> Reverse primer for PCR amplification of NGA162 SSLP marker in
Arabidopsis thaliana subspecies

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ctctgtcaact cttttctctct gg

22

<210> 72

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<221> Forward primer for PCR amplification of NGA6 SSLP marker in
Arabidopsis thaliana subspecies

<400> 72
tggatttctt cctctcttca c 21

<210> 73
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Arabidopsis thaliana subspecies

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atggagaagc ttacactgat c 31

<210> 74
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<220>
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Arabidopsis thaliana subspecies

<400> 74
aatgttgtaa tccctctctc 20

<210> 75
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Arabidopsis thaliana subspecies

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tgatgtctctc tgaacaaga gc 22

<210> 76
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 Arabidopsis thaliana subspecies

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gagggcaaat ctttatctcg g

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<210> 77
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tggcttctcg ttataaacat cc

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 in Arabidopsis thaliana subspecies

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gogaaaaaac aaaaaaatcc a

21

<210> 79
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 in Arabidopsis thaliana subspecies

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cgacgaacccg acagaattag g

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<210> 80
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 Arabidopsis thaliana subspecies

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gaaatccaaa tccagagag g

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<210> 81
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 Arabidopsis thaliana subspecies

<400> 81

ctctcccact agtttttgtt cc

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<210> 82
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 Arabidopsis thaliana subspecies

<400> 82

taccgtcaat ttcacggcc

19

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ggatccctaa ctgtaaaatc cc

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<223> Forward primer for PCR amplification of CA72 SSLP marker in *Arabidopsis thaliana* subspecies

<400> 84

aatcccagta accaaacaca ca

22

<210> 85

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<223> Reverse primer for PCR amplification of CA72 SSLP marker in *Arabidopsis thaliana* subspecies

<400> 85

cccagttctaa ccacgaccac

20

<210> 86

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<223> Forward primer for PCR amplification of NGA151 SSLP marker in *Arabidopsis thaliana* subspecies

<400> 86

gttttcgggaa gttttgctgg

20

<210> 87

<211> 24

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<223> Reverse primer for PCR amplification of NGA151 SSLP marker in
Arabidopsis thaliana subspecies

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cagtctaaaa gcgagagtat gatg

24

<210> 88

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<223> Forward primer for PCR amplification of NGA106 SSLP marker in
Arabidopsis thaliana subspecies

<400> 88

gttatggagt ttctagggca cg

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<210> 89

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<223> Reverse primer for PCR amplification of NGA106 SSLP marker in
Arabidopsis thaliana subspecies

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tgccccatcc tgttttttc

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<210> 90

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<223> Forward primer for PCR amplification of NGA139 SSLP marker in
Arabidopsis thaliana subspecies

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agagccacca gatccgatgg

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<210> 91

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 Arabidopsis thaliana subspecies

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ggcttcggtt cactatccag g

21

<210> 92
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 Arabidopsis thaliana subspecies

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ggagaaaatg tcactctcca cc

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<210> 93
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 Arabidopsis thaliana subspecies

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aggcatggga gacattttag

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<210> 94
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 in Arabidopsis thaliana subspecies

<400> 94

ctccaccaat catgaaaatg

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<210> 95
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tgatgttgat ggagatggtc a 21

<210> 96
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 <223> Forward primer for PCR amplification of NGA129 SSLP marker in *Arabidopsis thaliana* subspecies

<400> 96

tcaggaggaa ctaaagtga g 22

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cacactgaag atggtcttga g 22

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/EP98/06977 (22) International Filing Date: 9 October 1998 (09.10.98) (30) Priority Data: PO 9745 10 October 1997 (10.10.97) AU (71) Applicant (for all designated States except US): RHONE-POULENC AGRO [FR/FR]; 14/20, rue Pierre Baizet, F-69009 Lyon (FR). (72) Inventors; and (75) Inventors/Applicants (for US only): DOUTRIAUX, Marie-Pascale [FR/FR]; 64, route de Villebon, F-91160 Saulx les Chartreux (FR). BETZNER, Andreas, Stefan [AU/AU]; 40 Dallachy Place, Page, ACT 2614 (AU). FREYSSINET, Georges [FR/FR]; 21, rue de Nervieux, F-69450 Saint Cyr au Mont d'Or (FR). PEREZ, Pascal [FR/FR]; 17, chemin de la Pradelle, Varennes, F-63450 Chanonat (FR). (74) Agent: GENIN, Patrick; Rhône-Poulenc Agro, DPI, 14/20, rue Pierre Baizet, F-69009 Lyon (FR).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 24 June 1999 (24.06.99)
(54) Title: METHODS FOR OBTAINING PLANT VARIETIES (57) Abstract An isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant.		

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/06977

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 C12N15/29 C07K14/415 C12N15/10 C12N5/04
C12N5/14 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Date of the actual completion of the international search

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